Cannabis Science Task Force Recommendations

Cannabis Laboratory Quality Standards and Proficiency Testing

By

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For the

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Cannabis Science Task Force Steering Committee documents and recordings[4]

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[1] https://apps.ecology.wa.gov/publications/SummaryPages/1903004.html
[2] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html
[3] https://ecology.wa.gov/CannabisLabs
[4] https://www.ezview.wa.gov/site/alias__1962/37551/cannabis_science_task_force.aspx
[5] https://ecology.wa.gov

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- Joan LeTourneau
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The Washington State Department of Ecology (Ecology) prepared this report to the Legislature on behalf on the Cannabis Science Task Force (Task Force) as required by RCW 43.21A.735, which states:

> "(6) The cannabis science task force must submit a report to the relevant committees of the legislature by July 1, 2020, that includes the findings and recommendations for laboratory quality standards for pesticides in plants for marijuana product testing laboratories. The report must include, but is not limited to, recommendations relating to the following:
>
> (a) Appropriate approved testing methods.
>
> (b) Method validation protocols.
>
> (c) Method performance criteria.
>
> (d) Sampling and homogenization protocols.
>
> (e) Proficiency testing.
>
> (f) Regulatory updates related to (a) through (e) of this subsection, by which agencies, and the timing of these updates."
>
> "(8) Following development of findings and recommendations for laboratory quality standards for pesticides in plants for marijuana product testing laboratories, the task force must develop findings and recommendations for additional laboratory quality standards, including, but not limited to, heavy metals in and potency of marijuana products.
>
> (a) The cannabis science task force must submit a report on the findings and recommendations for these additional standards to the relevant committees of the legislature by December 1, 2021.
>
> (b) The report must include recommendations pertaining to the items listed in subsection (6)(a) through (f) of this section"

Executive Summary

The legalization of cannabis use in Washington resulted in the availability of cannabis[6] products throughout the state. Although cannabis products are required to be tested for harmful substances and potency, the science needed to develop adequate testing protocols has not caught up to the industry. The absence of a central scientific authority to develop and maintain cannabis-specific testing protocols and scientific processes has resulted in disparate and varied protocols and practices. It is crucial to establish and maintain science based processes to generate reliable data to support policy decisions and to maintain public trust.

In 2019, the Washington State Legislature made a positive step towards reconciling this effort in the creation of the Cannabis Science Task Force (CSTF), a multi-agency and industry scientific collaboration. Under the provisions of RCW 43.21A.735(6), the CSTF developed laboratory quality standards, making the first recommendations for standards for pesticide testing in plants and products in a July 2020 report. With those recommendations, the development of an Interagency Cooperative Team (ICT) was identified as crucial to act in a scientific regulatory leadership role (i.e., to oversee the implementation, facilitation, and maintenance of the cannabis laboratory quality standards).

In this second report, the CSTF provides comprehensive recommendations for additional laboratory quality standards covering potency, heavy metals, and residual

[6] The term "cannabis" is used throughout this document for > 0.3% delta-9 tetrahydrocannabinol-containing cannabis plants and/or materials.

solvents. Companion recommendations address ICT roles and responsibilities needed to provide initial and ongoing oversight and guidance for adopting laboratory quality standards and an in-state Cannabis Matrix Proficiency Testing Program. Finally, robust recommendations for developing this Proficiency Testing Program, including the identification of critical cannabis matrix proficiency testing samples, are presented. The in-state cannabis matrix proficiency testing pathway is critical to (1) ensure consumer protections and (2) meet the accreditation conventions needed to facilitate Washington State Department of Ecology's (Ecology's) accreditation model, as directed in RCW 43.21A.736.

Recommendations

To provide critical guidance to cannabis testing laboratories, and for Ecology to begin the accreditation of cannabis laboratories, several actions are needed. With the recognition that RCW 69.50.587 states that the Liquor and Cannabis Board may adopt rules that address the findings and recommendations[7] in the task force reports provided under RCW 43.21A.735, the Cannabis Science Task Force proposes that the following actions be implemented:

1. Washington State departments of Agriculture (WSDA), Health (DOH), and the Liquor and Cannabis Board (WSLCB) initiate and complete statute changes for formation of an Interagency Cooperative Team (ICT).
2. WSLCB initiates and completes statute and rule changes to facilitate an in-state Cannabis-Matrix Proficiency Testing Program.

   At the time of this publication, the WSLCB has regulatory authority over possessing, transporting, production, processing, and retailing of cannabis.
3. The ICT finalizes laboratory quality standards recommendations and advances the in-state Cannabis Proficiency Testing Program.
4. A regulatory agency with appropriate authority adopts laboratory quality standards for approved testing methods, method validation protocols, method performance criteria, sampling[8] and homogenization protocols, as well as proficiency testing.

   At the time of this publication, the WSLCB is the regulatory agency for laboratory quality standards. The WSLCB and WSDA have proposed joint agency request legislation for the 2022 Session to transfer this authority to the WSDA.
5. Ecology updates its existing proficiency testing guidance and accreditation rule to accommodate cannabis testing laboratory criteria.
6. Accreditation authority is transferred to Ecology following implementation of the proposals outlined above.

[7] Task Force recommendation motions are presented in Appendix A.

[8] "Sampling" refers to in-laboratory practices only; this is commonly termed "sub-sampling". Lot and batch sampling, as specified in WAC 314-55-101, falls outside of the scope of laboratory quality standard updates provided by the Task Force.

Introduction

Background

On July 28, 2019, RCW 43.21A.736 and RCW 69.50.348 became effective and set the authority and responsibility for cannabis testing laboratory accreditation requirements to transfer from the Washington State Liquor and Cannabis Board (WSLCB) to the Washington State Department of Ecology (Ecology) on July 1, 2024. The transfer of laboratory accreditation authority places cannabis testing labs under Ecology's well-established accreditation model; this model is based on standards being established by other entities. This transfer helps ensure labs are capable of providing accurate and defensible analytical data when implementing required laboratory standards.

RCW 43.21A.735 also became effective on July 28, 2019, which established a Cannabis Science Task Force (CSTF) to develop recommendations for meaningful science-based practices for cannabis lab testing. The CSTF, a multi-agency and industry scientist collaboration, was directed to develop recommendations for appropriate cannabis laboratory quality standards. These recommendations establish requirements for cannabis testing labs to follow and for Ecology to use to verify that labs are capable of generating accurate and defensible data. The WSLCB may adopt rules that address the findings and recommendations in the CSTF reports.

The CSTF submitted the first of two required reports to the Legislature in July 2020. This 2020 report, *Cannabis Science Task Force: Laboratory Quality Standards for Pesticides in Cannabis Plants and Products*[9] (Sekerak, 2020), detailed the recommendations to strengthen the testing protocols for pesticides in cannabis plants and compliant intermediate products. The CSTF recommended:

- Using existing agricultural method validation protocols and method performance measures developed by the United States Department of Agriculture (USDA), adapted to cannabis plants and products.
- Establishing an Interagency Cooperative Team (ICT) staffed by WSLCB, WSDA, and DOH to maintain the adopted protocols and provide technical assistance to cannabis laboratories.
- Performing timely and coordinated regulatory updates to facilitate these recommendations.

Deliverables (RCW 43.21A.735) for this second report to the Legislature include recommendations for (1) testing methods and protocols for potency, heavy metals, residual solvents, and proficiency testing (PT), and (2) preliminary guidance on microbiological testing. Additional provisions and groundwork for the ICT and Cannabis-Matrix PT Program are outlined. Included in the discussion is CSTF framework and process, recommendations to address gaps and challenges, tangential issues outside the CSTF scope yet highly important to the success of the cannabis testing industry, and finally an implementation plan for regulatory updates leading to transfer of accreditation to Ecology.

[9] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html

Cannabis Science Task Force

Revised Code of Washington 43.21A.735 established a Cannabis Science Task Force (CSTF) chaired by the state Department of Ecology (Ecology) and consisting of agency appointees from the state departments of Agriculture (WSDA) and Health (DOH), as well as the state Liquor and Cannabis Board (WSLCB). Participation by tribal and industry scientists was also a crucial piece of the CSTF design. See Appendix C for the complete list of appointed CSTF members.

The central goal of this multi-agency and industry scientist collaboration was to define and recommend reliable science-based practices for cannabis lab testing. The CSTF Steering Committee met at least monthly from August 2019 through mid-June 2021 to formalize laboratory quality standard recommendations. Twenty-four public meetings were held during that period.[10]

As displayed in Table 1, eight workgroups were formed to research and provide initial recommendations for all current cannabis testing fields and proficiency testing (PT). The recommendations were further refined into the formal laboratory quality standards and PT provisions that follow in this report.

Table 1. Workgroups focus areas, tenures and status, and location of final cannabis laboratory quality standards and proficiency testing recommendations.

Workgroup/ Focus Area	Term	Status	Location of Final Recommendations
Analytical (Pesticides)	August 2019 – February 2020	Completed	Report #1 [C]
Proficiency Testing	August 2019 – March 2020	Completed	Report #2 [D]
Heavy Metals	June 2020 – February 2021	Completed	Report #2
Potency	June 2020 – June 2021	Completed	Report #2
Residual Solvents	March 2021 – June 2021	Completed	Report #2
Microbiological	April 2021 – 2022	Continuing	Report #2; Memo to WSLCB/ICT
Mycotoxins [A]	June 2021 – 2022	Continuing	Memo to WSLCB/ICT
Moisture Content and Water Activity [B]	June 2021 – 2022	Continuing	Memo to WSLCB/ICT

[A] Continuation of most members from Residual Solvents Workgroup.
[B] Continuation of most members from Potency Workgroup.
[C] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html
[D] This current report.

[10] Beginning in March 2020, the Task Force public meetings were held virtually; however, the meetings still remained open to the public.

Work continuation

Early on, the Cannabis Science Task Force committed to completing all cannabis laboratory quality standards during its tenure. This commitment went beyond the minimal requirements for pesticides, potency, and heavy metals specified in RCW 43.21A.735. All recommendations finalized by June 2021 are contained in this second report to the Legislature. Workgroups will continue to develop recommendations through early 2022, or as funding resources[11] permit, for the laboratory quality standards that require additional time.

Starting June 2021, existing workgroups were assigned new laboratory standard focus areas to complete the CSTF commitment. Those workgroup focus areas are identified as “continuing” in Table 1. The workgroup that completed the potency standards shifted their efforts to the development of laboratory standards for moisture content and water activity. The Residual Solvents Workgroup transitioned to development of standards to address mycotoxins by liquid chromatography mass spectrometry. The workgroup already deliberating on microbiological recommendations will continue its work towards (1) completion of standards for shiga toxin producing escherichia coli, salmonella, and enterobacteria, and (2) development of additional standards for mycotoxins by rapid methods, as time allows. Finally, the laboratory quality standard recommendations for foreign matter will be completed by any workgroup, as availability and time permits.

The expectations and deliverables of the workgroups to recommend comprehensive laboratory standards will remain the same as outlined in RCW 43.21A.735(6); however, the final CSTF deliverable will differ. Each group continues to investigate and recommend testing methods, validation protocols, performance specifications, lab sampling, homogenization practices, proficiency testing, and regulatory updates needed to facilitate the new standards for each ongoing topic. A final CSTF memo covering the remaining laboratory standard focus areas will include all collected recommendations. The memo will be submitted to WSLCB to house until the ICT, as recommended in the first report[12] and described further in this report, is formed.

Laboratory quality standards support product standards and accreditation standards

Consumer protections rely upon established product standards, laboratory quality standards, and accreditation standards (Figure 1). Adequate and up-to-date laboratory quality standards are necessary to support established product standards and also facilitate accreditation activities. Laboratory quality standards establish critical approved testing practices for labs to follow and provide essential information for accreditation to verify a lab is capable of conducting those specific testing practices. Accreditation standards provide a framework of activities and processes needed to assess lab competency. These standards outline the

[11] The transition to virtual meetings resulted in a cost savings that were redistributed to continue the tenure of the Task Force beyond the anticipated December 2021 end date.
[12] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html

established accreditation cycles and on-site audit frequencies, application requirements, fee structures, and other procedural practices.

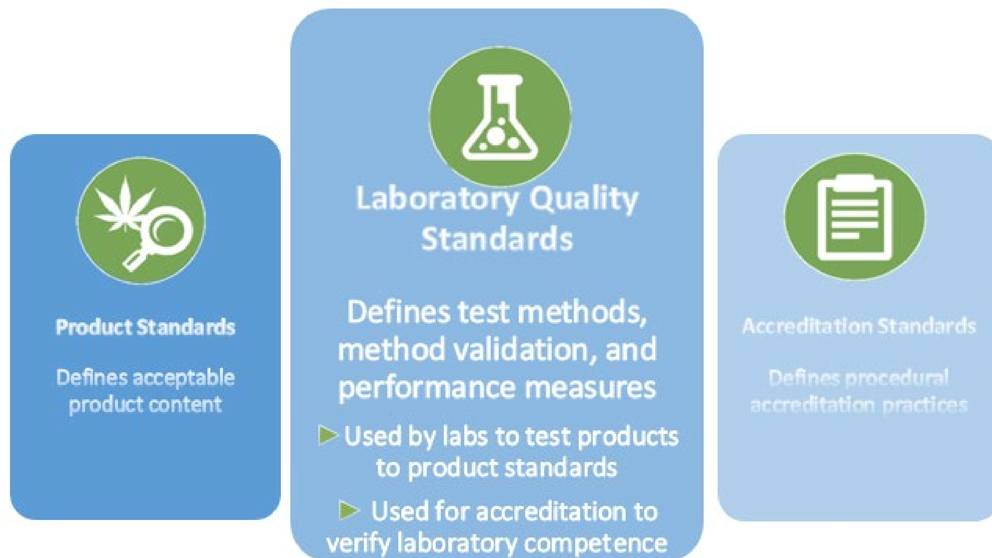

Figure 1. Product standards, laboratory quality standards, and accreditation standards.

Defining standardized methods and performance-based methods

The Cannabis Science Task Force explored both standardized methods and performance-based methods when formulating recommendations for laboratory quality standards for cannabis testing. To frame and support method recommendations found in subsequent sections, an explanation of each method premise is needed.

Standardized methods

Standardization can be simply described as the process of making something conform to a standard. In regulatory laboratory sciences, standardization of methods and processes impose specific design elements for consistency, reliability, repeatability, quality, and accuracy. This ensures that the methods produce a comparable output, regardless of who implements the method. Often referred to as prescriptive methods, this type of methodology is carefully designed; the prescribed process undergoes rigorous testing, validation, and peer review steps to achieve a specific goal and scope of work. The method designers, often regulatory or consensus bodies, stipulate goal-specific quality assurance and quality control (QC) measures to ensure adequate method performance and that the data generated is usable for its intended purpose. To maintain the original method's integrity, modifications by the end users are not customarily allowed, as modifications bring in new variables and potential sources of error that were not accounted for or validated in relation to the original method's goal and scope.

These methods require labs to implement specific types of instruments, chemicals, or standards prescribed in the method. Conversely, standard or prescriptive methods help outline the operational and start-up cost expectations for new labs wanting to implement a method. Standardized methods are often implemented in higher risk situations, such as testing against human health action limits. As an example, the use of prescriptive methods is required to test drinking water. Notably, the CSTF potency method recommendations followed this premise.

Performance-based methods

The alternative to standardized methods is the performance-based methodology system. The premise allows for each lab to independently design new methods and self-validate those methods. The performance-based methods system relies not on a single method, but rather a structure that provides a series of guidelines and protocols for method validation and method performance. Method validation protocols commonly outline how to perform the validation and how much and what kind of data is needed to demonstrate the method is suitable for its intended purposes. Method validation activities include demonstration of a method's performance characteristics for accuracy, precision, sensitivity, selectivity, limit of detection, limit of quantitation, linearity, range, and ruggedness. Additional required initial or ongoing performance measures may include requirements for the implementation of quality assurance schemes or that methods include specific quality control measures.

While this premise allows for maximum method design flexibility for the lab, the system has additional requirements including an active central oversight entity independent from accreditation to (1) review each unique laboratory method and accompanying validation data

and (2) authorize use of each method. Performance-based methods are often used in lower risk situations, such as trend monitoring, or in programs where quality systems are already well established. For example, the CSTF's 2020 report discusses the pesticide recommendations that are built around performance-based methods adapted from the USDA's extensive quality systems.[13]

Importance of in-matrix proficiency testing

Proficiency testing (PT) serves an integral role in regulatory laboratory testing programs. PT is a means of evaluating a lab's performance under controlled conditions relative to a given set of criteria through analysis of unknown samples provided by an external source.[14] The key to a successful PT program is that PT assessment samples adequately represent real everyday samples. This means PT samples containing the analytes of interest, at comparable concentrations, and in matrices matching and of equal complexity to the samples tested daily. PT samples are designed to test a lab's established analytical methodology, quality assurance system, and personnel employed in normal testing activities.

PT assessments serve as one of the main pillars of accreditation by providing valuable information for auditors to assess competency of a lab. PT assessments also can be a constructive tool for laboratories as a driver to (1) improve in-house performance, (2) identify "near-misses," (3) make corrections to laboratory methods and procedures before failures occur, and (4) provide periodic learning experiences for the lab testing personnel.

To be an effective resource for accreditation and genuine tool for labs, appropriately matrix matched or in-matrix PT samples are a necessity. If everyday samples produce complex challenges during testing, an adequate PT sample must be similarly complex. Independent PT provider schemes develop in-matrix PT samples to resemble an industry's everyday tested samples, both chemically and physically. Proficiency test samples are to be tested according to everyday implemented processes and should elicit the same instrument response. This premise helps to validate that the methods, processes, and quality control employed by a lab truly can meet the analytical requirements and produce accurate data when put to a blind test. Adequately employed PT schemes cover daily encountered sample matrix types, across the dynamic concentration ranges, for each analyte tested daily.

Generally speaking, one should easily see that plant materials physically look different from oils, or hard candy; therefore, it may also be easy to understand how breaking down these matrices might require different approaches. In fact, often drastically different preparation processes may be required to get each sample into the same injectable form needed for testing on the analytical instrument, even when being analyzed for the same chemical. For instance, a grinder might be initially used for plant or solid samples, but not for oil or liquid samples. In

[13] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html
[14] Proficiency Testing as defined by the National Environmental Laboratory Accreditation Conference (Program): https://nelac-institute.org/docs/2003nelacstandard.pdf

these cases, multiple matrix-specific PT samples are most appropriate to test the array of implemented preparation routes established in methods used daily.

Chemically speaking, it should be expected that plant, oil, and hard candy sample matrices are different as well. Each of these forms contains a complex mixture of chemicals responsible for how they uniquely look, taste, smell, and feel. Further processing of plant materials to produce other consumable forms causes chemicals to transform, interact, become removed or concentrated, or mixed with other complex chemicals, thus vastly altering the chemical makeup. These varying chemical complexities can each present unique challenges for analytical testing, which emphasizes the importance of matrix-specific PT samples.

Powerful tool for quality control

Analytical testing methods are constructed to mitigate effects attributed to natural and manufactured chemical compositions in order to test for a target element or compound. Consequently, some testing methods may have a limited scope of sample matrix compositions they can test. Other methods may be dynamic and well suited to test a wide scope of chemically divergent matrices. Routine testing can nonetheless be further complicated by the presence of or concentration levels of non-targeted or background chemicals that obscure, enhance, or alter the targeted analyte and the ability to accurately detect it. Daily QC designed into methods helps to monitor if a method performs adequately under these circumstances. However, participation in well-designed in-matrix PT assessments provides a vital comprehensive quality control test, beyond what daily controls are designed to do.

Testing PT samples that closely represent real samples is a powerful QC tool to help auditors and labs alike to delineate where one method or process does not function adequately or is generating inaccurate data. Results from PT assessments may show that a method’s techniques or daily QC practices are not adequately in place or implemented properly. Implementation of in-matrix PT assessments can help indicate when methods perform differently when interfering chemicals are present in one matrix type but not in alternate sample matrices. PT assessments can help reveal when methods are not dynamic enough or consistently capable of testing a broad array of divergent matrix types. When more types of everyday matrices are represented in PT assessments, more information can be gathered to better indicate competence in a lab’s methodology applied to everyday testing.

While it may not be practical to test all possible everyday tested sample matrices, categorical breakdowns of matrices are commonly implemented. Matrix categories capture the unique physical and chemical characteristics and complexities. For instance, regulatory environmental testing programs often break down matrix categories into non-potable waters, soils or other solid materials, tissues, and drinking water. Throughout the environmental testing industry, it is understood that a water PT sample is not sufficient to represent the complexities presented in testing solid materials. This is more evident when characteristic complexities are drivers for the creation and use of different methods to adequately support data uses, such as making policy decisions or protecting human health. Varying by industry, purpose of testing, and how data are used, federal and state authorities, and the industry itself, may all require matrix representative PT to support both policy and best scientific practices.

Barriers to cannabis (in-matrix) proficiency testing

As discussed at length in CSTF steering meetings, many challenges and barriers prevent the availability of adequate in-matrix proficiency testing (PT) samples to cover the legalized cannabis testing industry. Unlike other testing industries, adequate PT samples for the cannabis testing industry are not available due to the federal legal status and prohibition on interstate transport of cannabis and cannabis products. While some PT samples exist in the industry, there is a limited scope of types of PT samples available, and all types are missing one key component that sets this industry apart from others: the presence of delta-9 tetrahydrocannabinol (THC) that is representative of daily tested legalized cannabis samples (THC > 0.3%). As a key compound, THC is not only responsible for matrix complexities affecting various testing parameters, but it is also a target compound requiring testing itself.

Since federal law prevents the transport of cannabis across state lines, widely recognized third-party PT providers could only offer cannabis testing PT Studies with "surrogate" or non-cannabis matrix PT samples. As an interim solution, these PT Study designs were adopted by Washington and other states developing cannabis testing programs. The interim remedy offered the cannabis industry the PT premise that is used by other regulatory testing programs; however, the remedy did so only in principle, as testing *non-cannabis* matrices is not truly sufficient for determining proficiency and capability of testing *cannabis* matrices.

This challenge is not unique to Washington; other states' cannabis programs face the same federal hurdle impeding in-matrix PT. Some states have begun to formulate and implement solutions, as the need for in-matrix PT becomes evident. States such as Oregon and Colorado have made provisions allowing a credentialed[15] PT provider to obtain licenses or otherwise be permitted to buy, possess, manufacture, transport, and sell cannabis PT samples. In these schemes, a credentialed PT provider can come into a state, source cannabis and cannabis products from inside of that state, and manufacture and distribute vital cannabis-matrix PT samples to regulatory testing labs. Other states, such as Nevada, are also actively pursuing in-matrix PT schemes.

For Washington certified labs, proficiency tests are currently required; however, they are performed in other surrogate matrices (e.g., hemp flower and hemp-based matrices). Statute and rules have been identified as barriers to the implementation of a cannabis-matrix PT scheme in Washington. These barriers include, but are not limited to, legal possession provisions, licensing requirements for transporting cannabis materials, the traceability of cannabis moving through the state, and the sale of cannabis materials in Washington.

[15] Compliant in Organization for Standardization (ISO) /International Electrotechnical Commission (IEC) 17043 and ISO 17034, for competency of providers of proficiency testing schemes, as well as competency for the manufacture of reference materials and PTs, respectively.

Coordinated actions and accreditation transfer

The comprehensive recommendations in this report should be adopted to facilitate a robust cannabis testing program in Washington. The recommendations portray carefully constructed and deliberate laboratory quality standards that instruct labs on what to test for and how to test, as well as what quality of testing supports the current product standards and is required for Ecology's accreditation to commence. Intrinsically tied to these laboratory quality standard recommendations are recommendations for (1) the formation of an ICT to provide scientific oversight and (2) development of an in-state cannabis (in-matrix) proficiency testing (PT) program. Together all components are critical to the future success of legalized cannabis testing in Washington.

Coordinated actions and timely implementation are needed for a seamless transfer of cannabis laboratory accreditation (Figure 2). Laboratory quality standards and their companion recommendations established in statue, rule, or guidance will allow Ecology to amend its rules and guidance (Chapter 173-50 WAC).

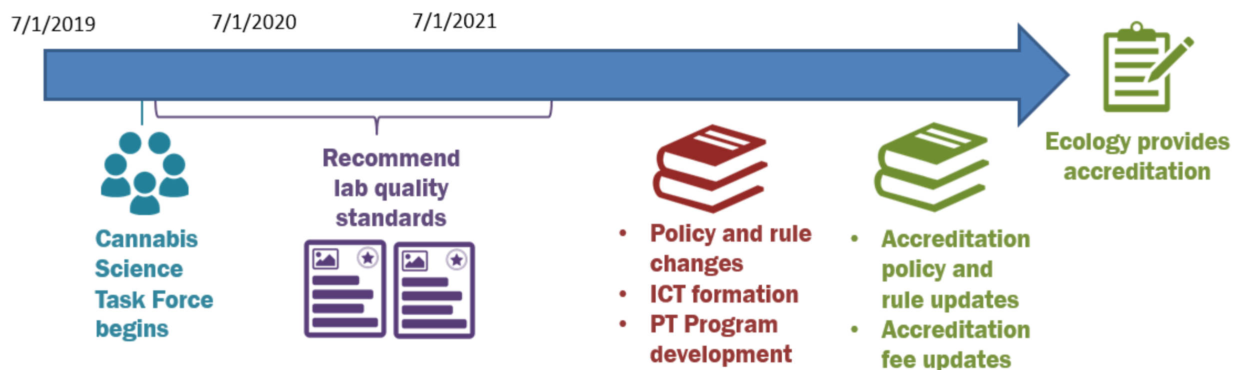

Figure 2. Cannabis Science Task Force tasks and needed implementation steps.

Interagency Cooperative Team

In addition to recommending testing methods and protocols, the first CSTF report to the Legislature[16] in 2020 initially identified the critical need for an Interagency Cooperative Team (ICT) to establish, facilitate, maintain, and provide guidance for those performance-based methods and protocols. Staffed by WSLCB, WSDA, and DOH, this multi-agency body would hold scientific subject-matter expertise in chemistry and microbiology, food and agricultural testing, pesticide testing, and other lab testing practices.[17]

To fill the gap left by an absence of federal oversight, the ICT would provide much needed scientific oversight and ensure alignment of Washington's cannabis policy with practical, and evolving, scientific activities. This essential and integral leadership role must ensure that (1) policies for the correct type, quality, and quantity of data collected are in place and (2) data are suitable to support consumer protections and future policy decisions. Notably, Colorado and more recently California[18] have moved to merge their states' various scientific discipline authorities to provide a more robust centralized scientific cannabis oversight within those states.

Since drafting the 2020 report to the Legislature, the CSTF has identified additional roles and responsibilities that need to be carried out by a scientific oversight team, which further supports the formation of the ICT as Washington's own scientific oversight authority for cannabis testing practices.

Genesis of the ICT

A prominent element leading to the formation of the CSTF was that appropriate methods, protocols, and oversight for testing cannabis were lacking in Washington State. Further, a federal agency that normally would provide cannabis-specific scientific oversight and testing practices, such as the USDA does for agricultural testing or EPA does for environmental testing, is absent. This significant absence is not only adversely affecting cannabis testing in Washington, but also in the entire legalized cannabis testing industry. Therefore, upon formation, the CSTF was propelled into filling an interim role of scientific oversight for assessing, adapting, and recommending the best available science practices from other

[16] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html

[17] Serving as an arm of government that establishes cannabis testing regulations, it is necessary for this ICT to remain separate from both those that accredit to the regulations and the laboratories that perform under the regulations. Ecology, serving as the accreditation authority, must remain separate to maintain the third-party accreditation premise enabling them to conduct objective assessments of conformity to the testing regulations. Additionally, modeling after other regulatory testing oversight bodies, members of the public and those being regulated would not serve as members of the ICT.

[18] In July 2021, California formed the Department of Cannabis Control thereby consolidating the Department of Cannabis Control, Department of Food and Agriculture, and the State Department of Public Health into one department to advance efforts to streamline the state cannabis regulatory structure. Scoped into their strategic plan is the creation of a state-run cannabis laboratory. https://cannabis.ca.gov/2021/07/13/governor-newsom-signs-cannabis-trailer-bill-creating-the-department-of-cannabis-control/

established federal and state regulatory programs to apply to cannabis testing in Washington State.

In 2019, to address the best testing practices for pesticides in cannabis plant materials, the CSTF looked toward leveraging widely accepted performance-based agricultural methods and protocols from the United States Department of Agriculture (USDA). As the CSTF moved towards recommending existing USDA protocols and performance measures for cannabis testing, the need of a role to provide ongoing scientific oversight became a significant topic.

Oversight roles and relationships that exist between the USDA and their partner state agricultural laboratory divisions, including the WSDA, were presented to exemplify how other regulatory testing programs function. The terms "client"[19] and "data-user" were used to describe the partnership that exists between the WSDA and USDA. Specifically, as outlined in the USDA's Pesticide Data Program (PDP), the USDA, serves in the role of the "client," and establishes and outlines the pesticide testing specifications in policy and published PDP standard operating procedures (SOPs). The WSDA then performs the testing of agricultural commodities to the USDA's policy specifications and follows the USDA's established SOPs.

As outlined in those SOPs, USDA technical experts serve to (1) monitor PDP program compliance and field technical questions, and (2) provide essential method guidance and method validation reviews for State testing entities such as the WSDA. These technical experts include the Monitoring Programs Division (MPD) Director, MPD Technical Program Manager, and Quality Assurance Officer.

When the CSTF formally adopted the USDA PDP SOPs as pesticide laboratory quality standards for cannabis testing, a companion recommendation was needed to establish an oversight entity. A second recommendation followed for the formation of an Interagency Cooperative Team (ICT) to emulate the USDA client role for ongoing oversight. The recommendation also stipulated that the ICT must possess appropriate authority and expertise to maintain all of the CSTF pesticides proposals. As detailed in the first CSTF report to the Legislature, the ICT must also help finalize laboratory quality standards and ensure adequate placement in rule or guidance in order to facilitate all sequential actions needed for the transfer of accreditation authority.

Additional ICT roles and responsibilities[20]

Every workgroup convened identified both basic scientific process and day-to-day cannabis product testing challenges where scientific oversight and guidance was needed. Scientific oversight for regulatory laboratory testing programs is essential to ensure adequate laboratory practices are established and implemented appropriately in order to produce data consistent across all labs that are the right type, quality, and quantity to make well-informed decisions.

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[19] The term "Interagency Cooperative Team" or "ICT" replaces the multiagency cannabis scientific team identified as the "Client" in the 2020 Legislative report.

[20] Anticipated statue and rule updates necessary to facilitate these roles and responsibilities are discussed in the "Recommendations" section of this report.

The ICT should possess the capability to provide ongoing technical assistance for all approved cannabis testing methods and practices. As described in the 2020 legislative report, the ICT should be comprised of subject-matter experts in chemistry and microbiology, food and agricultural testing, pesticide testing, and other laboratory testing practices. As conceptualized, the ICT would fill the gap of scientific oversight of legalized cannabis testing that is normally filled by federal agencies, such as the USDA or FDA, that are not providing this oversight.

Adequate oversight and guidance is needed for method interpretation, facilitating performance and validation activities, guiding ongoing testing and sample challenges, and progressing the science to accommodate a dynamic industry and evolving testing technology. Specifically, when new methods are adopted and future updated versions of adopted methods are released, the ICT would provide method guidance on interpretation and implementation of the new method practices. ICT evaluations of methods, performance data, and validation data would be needed to ensure performance-based, lab-designed methods include all required standard components and meet the initial performance criteria. Ongoing guidance should also be available for lab sample challenges, such as received samples that are not adequate for testing by the approved methods. Finally, the ICT should be responsive to the changing cannabis industry and advancing technology, and also should progress the science and policy accordingly.

Collaboration between science and policy decisions should exist, especially when changes to companion rules may adversely impact successful implementation of established laboratory standards. When updating product rules, such as lowering product contaminant action limits or adding new contaminants or compounds, the ICT should be part of the earliest discussions. It is imperative that those with authority and scientific subject matter expertise carefully review and ensure that the previously established testing requirements remain appropriate and useable to accommodate changes to product standards. For example, if a previously approved method is not sensitive enough (i.e., not capable of detecting lower levels of contaminants at or near the new action limit), the ICT would need to select and authorize a new, more sensitive, testing method. Failing to address this proactively would lead to a laboratory standards gap and an inability to test products and produce valid testing data to support the new product rules.

The ICT technical experts within the oversight body should possess subject-matter expertise for daily technical support and also adequate scientific decision-making authority for advancing and supporting the science and science policy. The ICT should work closely with policy experts to help establish and make needed updates to laboratory standards in order to adequately support changes made to related cannabis standards, thereby collectively and cohesively providing better consumer protections.

Initial integration of laboratory quality standards

To assist with the integration of the CSTF recommendations as laboratory quality standards and prepare for the transfer of accreditation to Ecology, the timely formation of the ICT must occur to facilitate the following activities:

- Finalize and/or approve laboratory quality standard recommendations from the CSTF.

- Perform additional subject-matter led technical research needed to review and designate appropriate testing methods and practices (i.e., designating procedures for microbiology, mycotoxins, moisture content, water activity, and foreign matter).
- Author guidance or standard operating procedures (SOPs) as needed to facilitate practical use of laboratory quality standards. For example, as recommended in the 2020 legislative report, combine the Summary of Adaptations to the USDA PDP SOP Model document (2020; Appendix A) and the five USDA PDP SOPs (2020; Appendix B) into a client-written SOP or manual to facilitate ease of use.
- Provide subject-matter technical guidance and scientific reviews for new rule and guidance language and for updates to existing rule language.
- Provide chemistry and microbiology expertise for the cannabis-matrix proficiency testing (PT) program (e.g., scoping contract and reviewing bids for third-party PT providers, providing liaison duties, investigating PT quality issues).
- Prepare ICT procedures for performance-based method validation reviews as required by approved methods or processes (e.g., pesticides and others identified in CSTF final memo of continued work).
- Prepare for ongoing subject-matter specific technical support for the laboratories.

Ongoing methods maintenance

Once the ICT is established, subject matter experts should be available to the cannabis testing labs for day-to-day activities as well as for implementation of new or updated laboratory quality standards for all required fields of testing. This role serves to ensure that scientific rigor is practiced and that data produced is appropriate when used for enforcement and other ICT-defined purposes, such as health risk assessments.

Required methods that were originally developed by federal or state regulatory bodies will require the ICT to continually review, provide necessary adaptations, and adopt future revisions released by the primary regulatory authors. A process should be created to ensure new method versions, and any needed method adaptations, are able to be implemented in the testing labs in a timely manner. If these adaptation updates are provided in a guidance document, rather than in rule, the subject-matter experts in the ICT should initially assist in authoring and reviewing this guidance, as well as provide ongoing maintenance to ensure scientific rigor is upheld. In cases where the originating regulatory authors provide revisions to mitigate method problems, or necessary for consumer protections, swift action by the ICT would be needed. Rapid response in reviewing and authorizing method revisions would support extending the most up-to-date consumer protections to Washington.

Following authorization of new or updated required testing practices, the ICT should provide (1) overarching technical support, including interpretation and guidance for testing labs, and (2) any provisions noted in the following field-specific report sections. Additional interpretation and guidance should be provided to Ecology’s Laboratory Accreditation Unit in order for accreditation processes to account for and adapt to new or changing laboratory standards and testing expectations.

ICT Formation Framework

Beginning late spring 2020, personnel from the three identified partner agencies, WSLCB, WSDA, and DOH, began meeting to discuss the potential roles, responsibilities, and authority of the ICT. Leveraging off of the ICT recommendations submitted in the first CSTF legislative report[21] and additional content formalized in this second legislative report, the agency legislative directors, rules coordinators, and policy leads met periodically to hone the initial framework of the ICT. Progress updates were provided at each monthly CSTF meeting.

Since publishing the 2020 legislative report, the need for the scientific oversight team became more important. The ICT would need to facilitate initial adoption, provide ongoing maintenance, and create a pathway for future development of laboratory quality standards across all fields of testing. Roles and responsibilities for standards interpretation, implementation guidance, technical assistance, and validation and review activities were identified for the ICT throughout the testing fields examined. Also, the ICT is designed to be the scientific lead for developing and facilitating the in-state Cannabis Proficiency Testing Program. In fact, most of the CSTF recommendations are reliant, if not contingent upon, the adequate and timely formation of the ICT. This is to improve cannabis testing, build stronger consumer protections, and enable the accreditation transfer to Ecology.

While this report was being written, ICT framework development activities were re-scoped to more comprehensively address roles and responsibilities identified through the second half of the CSTF's tenure. Agency request legislation jointly sponsored by WSLCB and WSDA has been proposed for the 2022 legislative session.

Potency

For potency, the CSTF leveraged off methodologies developed by the New York State (NYS) Department of Health designed specifically for testing medicinal cannabis products (Appendix B). The recommendation for the adoption of these methods was deliberate to reduce risk, deliver better consumer protections, and provide for consistency and accuracy in analysis and accreditation. As with all regulatory required scientific methods, periodic revisions to the NYS methods are anticipated. Method updates are common to address regulatory changes and adjust for ongoing advancements in testing technologies, both of which should be anticipated due to the infancy and dynamic evolution of the cannabis industry. Additionally, the recent legalization of recreational cannabis in New York may lead to method updates to accommodate testing a wider scope of product types.

After establishing the potency laboratory quality standards for Washington, the ICT subject-matter experts should regularly monitor for and provide timely reviews to any NYS released method revision updates. The ICT reviews would determine applicability and appropriateness of the method updates, and recommend adoption when necessary. Review of the companion comprehensive potency adaptions (Appendix A, April 29, 2021) must be completed to ensure the new NYS method content is still in agreement with the previously established Washington-

[21] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html

specific adaptions. As appropriate, the ICT should update and adopt the newer versions of the Potency Adaptations to support the adoption of any new NYS method version. Additional reviews should be performed periodically, and when updates are necessary, for additional regulatory purposes.

In the future, it may become necessary to authoritatively expand and modify the standardized potency methods to increase the methods' ability to test a wider scope of Washington-specific products. For standardized methods, this type of scope change is usually performed by the originating regulatory agency lab scientists; however, specific Washington needs are unique and fall outside of NYS's updating obligations. Work leading to these modifications must be accomplished in a lab and must employ robust method development and validation processes to endorse and support the intended modifications. In the absence of a state-run cannabis lab to perform this work, the CSTF recommended that collaborative method development and validation studies could be performed by the accredited cannabis testing labs under guidance and oversight by the ICT. The CSTF provided an initial outline for the process; however, the ICT will still need to finalize the collaborative method modification study pathway.

In the continued development of this pathway, it was recommended that the ICT should initially review, make necessary adaptations, and adopt the U.S. Food and Drug Administration (FDA) document "Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products" and the "AOAC Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis" (Appendix B). The ICT should leverage FDA and AOAC (Association of Official Analytical Chemists) guidance to further build a framework with the capability and capacity to (Appendix A, April 29 2021 and May 24, 2021):

- Determine the purpose and scope of studies.
- Authorize a sponsor lab to begin initial work.
- Coordinate candidate labs for the collaborative (inter-laboratory) phase.
- Provide validation reviews and feedback on data generated by the candidate modified methods.
- Approve and authorize the use of modified methods, as appropriate.

The ICT oversight role may also determine that other modifications are needed to accommodate rule updates or trends in the legalized cannabis industry, such as adding additional cannabinoids to the method's scope. The same collaborative method modification study pathway should be capable of accommodating these additional method scope modifications.

Heavy Metals and Residual Solvents

To address heavy metals and residual solvents testing, the CSTF recommended the use of adapted EPA methods. The ICT should perform a method review of each subsequent EPA method version. This review should be completed prior to authorizing a new EPA-released method version. EPA method revisions do not occur often because the methods are well established. The EPA does periodically update methods to align with new policy or to include provisions for advances in science and technology. The ICT should conduct timely reviews of the

revision updates to ensure that methods still meet the methodology needs to support Washington's legalized cannabis regulations.

In addition, systematic and regular scientific reviews should be performed on the recommended method adaptations designated as validation criteria, performance protocols, lab sampling, and homogenization techniques that serve as additional laboratory quality standards. After updates to methods or method adaptations, the ICT may need to deliver method-specific technical assistance for the labs to implement updates or guidance in the practical development of laboratory techniques, just as EPA customarily provides for Ecology's testing lab.

Microbiology, mycotoxins, moisture, water activity, and foreign matter

As of June 2021, the CSTF did not conclude recommendations for approved methods, validation criteria, performance protocols, lab sampling and homogenization techniques for the remaining cannabis testing fields[22]. The continued efforts of the CSTF will aim to address the remaining laboratory quality standards for each field and provide a summary to the WSLCB to house until the ICT is formed. Recommendations provided outside of this report should be heeded in earnest, as they were developed with the same thorough considerations as those recommendations found in this report.

The ICT may still need to further develop these laboratory quality standards. The ICT must be formed in a timely manner and have the capacity to:

- Finalize and/or approve the final laboratory quality standard recommendations from the CSTF.
- Perform additional subject-matter led technical research needed to designate appropriate testing methods and practices.
- Author guidance or standard operating procedures as needed to facilitate practical use of laboratory quality standards.
- Provide subject-matter technical guidance and scientific reviews for new rule and guidance language and for updates to existing rule language.
- Provide chemistry and microbiology expertise for the Cannabis-Matrix Proficiency Testing Program.
- Prepare ICT procedures for performance-based method validation reviews as required by approved methods or processes.
- Prepare for ongoing subject-matter specific technical support for the laboratories.

[22] One microbiology recommendation was completed prior to the writing of this report. The recommendation provided only a premise for use of molecular-based testing for detecting Salmonella and pathogenic E. coli (Appendix A, June 11, 2021). The continued laboratory quality standards development will follow this recommended pathway.

Advancing the Science

It is expected that the expanding market will continue to produce new legal cannabis products. As more and more states move to legalize recreational use cannabis, trends within these legal-use states would presumably motivate trends in other legal states. As new products permeate state markets, the technology, methods, and standards for testing will need to evolve.

While the major instrumentation and technologies needed to test cannabis are largely already available due to use in other regulatory industries such as agricultural, environmental and other health sciences, more instrument manufacturers inevitably will shift into the cannabis testing platform. Customarily when designing for new and emerging contaminants of interest or matrices (e.g. product types), manufacturers begin to modify or market their existing instruments and testing techniques for those needs. In response, new industry-specific testing methods will be established or existing methods will be modified by consensus bodies, regulatory scientists, and other research scientists. For regulatory testing needs, rigorous frameworks and processes for method development are implemented to ensure that methods meet essential criteria for performance and quality.

As directed by RCW 43.21A.735, the CSTF looked at other jurisdictions that have established or are establishing cannabis testing programs for methods, validation and performance measures, and sampling and homogenization protocols. Currently 36 states and four territories permit medical use, and 18 states, two territories and Washington D.C. have enacted legislation allowing recreational use[23]. Many jurisdictions are or will be embarking upon the development of testing standards, including some with state-run cannabis laboratories performing much-needed cannabis testing method development. Those states' work could serve as valuable resources to Washington. In an effort to create a more unified national approach to cannabis testing, the ICT should look to other jurisdictions to continually monitor for and leverage the methods and processes developed by other cannabis-specific regulatory bodies.

Further, in the absence of both federal regulatory oversight and a state-run lab to perform method development for new technologies and methods, the ICT should facilitate a process to engage Washington's accredited cannabis testing labs for method development when accepted authoritative methods do not adequately cover the testing needs for Washington. The CSTF recommended an initial framework and process for method modifications for potency that could be further developed and formalized. This is to ensure adequate steps are included for verifying performance and quality, peer-review, and multi-laboratory method studies for all fields of testing. Both the FDA's Foods Program[24] and EPA[25] have already developed extensive chemical and microbiological method validation and peer review processes to ensure methods produce the correct type, quality, and quantity of data for making regulatory decisions and to protect human health. Their resources and processes may provide essential elements for the ICT to leverage.

[23] https://www.ncsl.org/research/health/state-medical-marijuana-laws.aspx

[24] https://www.fda.gov/food/laboratory-methods-food/foods-program-methods-validation-processes-and-guidelines

[25] https://www.epa.gov/measurements-modeling/method-validation-and-peer-review-policies-and-guidelines

Cannabis proficiency testing (PT) program facilitation

As one of the two initial workgroups required to be formed under RCW 43.21A.735, the Proficiency Testing (PT) Workgroup was responsible for the development of scientifically sound recommendations for PT.

Focusing on qualities of function, the recommendations for the design of an in-state cannabis (in-matrix) PT program were formulated to meet the following goals:

- Ensure PT samples serve to provide a proper mechanism to assess lab competency, offer quality feedback for labs, provide supplementary information for data users, and uphold consumer confidence.
- Ensure PT design includes adequate coverage for all cannabis parameters.
- Establish structure for PT samples to cover the wide-array of current and future cannabis and cannabis product matrices tested daily.
- Establish minimum requirements for third-party independent PT providers.
- Align regulatory testing for cannabis with other regulatory testing programs that require in-matrix PT.
- Establish representative cannabis matrix categories required for the implementation of Ecology's in-matrix accreditation model.
- Address accreditation processes and onboarding needed for the accreditation authority transfer.

The CSTF looked to other states implementing solutions to fill the critical in-matrix PT gap within the cannabis industry. In fall 2019, the CSTF specifically explored the cannabis in-matrix PT approach facilitated in Oregon and Colorado. In each of those states, a credentialed PT provider is permitted to come into the state, acquire cannabis and cannabis products, and importantly, manufacture and distribute in-matrix PT samples. Based on this research, one third-party PT provider[26] was invited to provide a presentation to the CSTF. The PT provider addressed design, issues, and implementation of their role in providing in-state in-matrix PT schemes.[27]

Through months of continued discussions, a proof-of-concept trial of an in-matrix PT study was conceptualized to test a similar plan in Washington (Appendix D). Unfortunately, the CSTF was unable to conduct the trial because of a myriad of barriers that couldn't be avoided. Further, where statute and rule held firm, it was ultimately realized that cannabis matrix PT would be stalled until those barriers were removed or adjusted.

The ICT could further explore the advantages and logistics of hosting an independent PT provider to service Washington's cannabis testing industry with vital in-matrix PT samples. The CSTF's proof-of-concept trial plan may be groundwork to help guide the ICT in collaboration

[26] At the time of the proof-of-concept study conceptualization, only one PT provider, Phenova Inc., was able to offer this in-state PT scheme service to Washington. Others third-party PT providers were contacted but did not respond to the Task Force's inquiry.

[27] https://www.ezview.wa.gov/Portals/_1962/Documents/CannabisSTF/PhenovaPTPresentation.pdf

with policy personnel to facilitate the creation of a Cannabis Matrix PT Program. Alternatively, and as also explored in the CSTF, a state entity, separate from accreditation, could become a PT provider to facilitate the necessary cannabis-matrix PT schemes. All PT recommendations support both options (see the Proficiency Testing Recommendations section for further details).

At a minimum, the ICT should serve to develop, facilitate, and maintain the following activities:

- Investigate PT quality issues of third-party providers.
- If the conditions are such that a third-party state run contract is required, the following recommendations are made:
    - Serve as the liaison between the labs and PT provider for items such as scheduling, transport, and quality concerns.
    - Develop the scope of contract(s) with a third party PT provider.
    - Review bids of contract(s) with a third party PT provider to ensure Ecology Laboratory Accreditation Unit, applicable RCWs/WACs, and CSTF requirements are met.

Laboratory Quality Standard Recommendations

The goal of the Cannabis Science Task Force (CSTF) recommendations is to provide (1) a science-based framework for testing laboratories to operate effectively and (2) appropriate information for accreditation to adequately determine whether a lab has the capability to provide accurate and defensible data. Together, these will build stronger consumer protections.

Laboratory quality standards are a critical element of consumer protection. Standards that are used in clinical, industrial, and food safety testing were developed over many years to ensure products are sufficiently tested to reduce the risk to consumers. Due to the short timeline for adopting cannabis lab quality standards into rule, the CSTF relied heavily on already approved standards, primarily from the agricultural and environmental testing industries. By doing this, the CSTF was able to leverage consumer protections built into these methods and protocols.

Testing methods, method performance criteria, and (in-laboratory) sampling and homogenization protocols are specific procedures that labs must use to achieve accurate credible data for regulatory purposes. Additionally, these provide the basis of accreditation activities. For instance, an auditor will observe a chemist completing a testing method to ensure they are following each step correctly.

As required in RCW 43.21A.735, laboratory quality standard recommendations must include designation of the following:

1. Appropriate approved testing methods
2. Method validation protocols
3. Method performance criteria
4. Sampling[28] and homogenization protocols
5. Proficiency testing
6. Regulatory updates

The second phase of deliverables outlined in RCW 43.21A.735 mandated the CSTF to develop recommendations for laboratory quality standards for heavy metals and potency, at a minimum. In addition to the mandated deliverables, recommendations for residual solvent laboratory quality standards as well as recommendations for a robust cannabis-specific proficiency testing (PT) program are included. These additional recommendations go beyond the original scope yet provide a more comprehensive set of guidance for rulemaking before the accreditation authority is transferred to Ecology.

The laboratory quality standards adopted by the CSTF are dependent on the formation of the Interagency Cooperative Team (ICT). Therefore, it is critical that law and rule updates allow for the formation of the ICT. This will ensure that the following CSTF recommendations are incorporated into rule or guidance for the cannabis laboratories to follow and for Ecology to accredit against. The ICT role in developing and facilitating the Cannabis Proficiency Testing

[28] "Sampling" refers to in-laboratory practices only; this is commonly termed "sub-sampling". Lot and batch sampling, as specified in WAC 314-55-101, falls outside of the scope of laboratory quality standard updates provided by the Task Force.

Program is needed to yield required in-matrix PT samples before accreditation authority is transferred to Ecology.

Potency recommendations

The Cannabis Science Task Force recommendations for potency are as follows[29]:

1. Appropriate approved testing methods: New York State (NYS) Department of Health (DOH) Medical Marijuana (MML)-301, revision 6 - Medical Marijuana Sample Preparation Protocols for Potency Analysis and NYS DOH MML-301, revision 6 - measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA (Appendix A, November 20, 2020 and Appendix B).
    a. When newer revisions of the approved test methods are published by the NYS DOH, the ICT must review the newest version(s) prior to implementation. Document control and ultimate revision decisions should be under the ICT authority. When new versions are authorized the laboratories would be required to update their methodology accordingly.
2. Method validation protocols: Method validation protocols are as established within each approved method.
    a. A future validation pathway for laboratory-initiated modifications to increase method scope, beginning with minor matrices/products not included in the NYS DOH MML methods, should be further developed by the ICT. The ICT pathway development must incorporate the key attributes (Appendix A, April 29, 2021 [CSTF Steering Committee Motion]), and further develop the process using outlined process guidelines (Appendix A, May 24, 2021). Guidelines include method validation and review using steps that require using the FDA document "Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products," Level 4 (Appendix B) and also "AOAC Appendix D: Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis" (Appendix B).
3. Method performance criteria: Performance criteria are as established within each approved method. The following additional performance measures and adaptations must supersede, or be used in conjunction with the minimum method requirements, as appropriate.
    a. The potency consensus adaptations to the NYS DOH MML – 301 Revision 6 and NYSDOH MML – 300 Revision 6 methods must be implemented to facilitate appropriate use of NYS methods (Appendix A, April 29, 2021).
    b. Potency determinations must be performed on samples "as is" or as received by the lab.
4. Sampling and homogenization protocols: Sub-sampling and homogenization protocols are as specified in the approved method(s).
5. Proficiency testing (PT): Potency PT must be performed on samples that are matrix matched or "in-matrix" to assess method performance. Cannabis matrix categories for PT

[29] The complete anthology of potency recommendations is compiled in Appendix A.

assessments will be established as flower, intermediates, and end products. (See Appendix A, October 28, 2020; and also Proficiency Testing Recommendation section.)

6. Regulatory updates: The appropriate regulatory authority must make timely regulatory updates to Washington Administrative Code (WAC) for the adoption and implementation of the recommended laboratory quality standards for testing laboratories and for transfer of accreditation authority to Ecology. Laboratory quality standard regulatory updates must be completed two years prior to the mandated accreditation transfer to Ecology. This will ensure Ecology can build its accreditation rules and guidance to adequately address the most up-to-date laboratory quality standard requirements. Updates to the current Ecology accreditation model will include updates needed for the addition of a cannabis accreditation fee structure and cannabis matrix categories. Earlier implementation of the laboratory quality standards could benefit laboratories and the current accreditation provider before the transfer of accreditation to Ecology.

   At time of this publication, the WSLCB is the regulatory agency for laboratory quality standards. The WSLCB and WSDA have proposed joint agency request legislation for the 2022 Session to transfer this authority to the WSDA.

   As noted in the 2020 CSTF legislative report, the WSLCB WAC 314-55-0995 language requiring laboratories to follow the Cannabis Inflorescence and Leaf Monograph published by the American Herbal Pharmacopoeia (AHP) must be removed. The AHP document cited in WAC 314-55-0995(3)(b) does not constitute an adequate set of laboratory quality standards for cannabis testing labs and accreditation. This specification must be removed to facilitate the recommendations for potency and all subsequent laboratory quality standards recommendations detailed in this report.

Heavy metals recommendations

The Cannabis Science Task Force recommendations for heavy metals are as follows[30]:

1. Appropriate approved testing methods: EPA SW-846 Method 6020B: Inductively Coupled Plasma Mass Spectrometry and EPA SW-846 Method 6010D: Inductively Coupled Plasma - Optical Emissions Spectrometry. Appropriate approved companion preparation methods include: SW-846 Method 3050B Rev. 2, Method 3052, and Method 3031 (Appendix A, October 28, 2020 and February 22, 2021, and Appendix B).
   a. When newer EPA method versions of the approved test methods or companion preparation methods are published, the newest version should replace the current method requirement and laboratories will be required to update their methodology accordingly. When the ICT is established, document control and ultimate revision decisions will be under their authority.

2. Method validation protocols: Method validation protocols are as established within each approved method.

[30] The complete anthology of heavy metals recommendations is compiled in Appendix A.

3. Method performance criteria: Performance criteria are as established within each approved method. The following additional performance measures and adaptations must supersede, or be used in conjunction with the minimum method requirements, as appropriate (Appendix A, March 23, 2021).
   a. Ultra high-purity or equivalent acids must be used in the preparation of standards and for sample processing.
   b. Analytical standards and solutions must be National Institutes of Standards (NIST) traceable or equivalent.
   c. Certified reference materials (CRMs) and/or standard reference materials (SRMs) are highly recommended when available for method development, troubleshooting and optional additional quality control (QC).
   d. Method-specified QC guidance should be followed; however, additional QC or changes to QC criteria may be set in addition to the minimum requirements.
   e. Instruments must be calibrated using a minimum of a four-point curve (no blanks can be used as a point). The correlation determination (r^2) should be ≥ 0.990 or the correlation coefficient (r) should be ≥ 0.995. Use Linear Regression with 1/x or no weighting. Forcing the curve through zero is not allowed.
4. Sampling and homogenization protocols: Sub-sampling and homogenization protocols are as specified in the approved method(s).
5. Proficiency testing (PT): Heavy metals PT samples should be performed in matrix matched or “in-matrix” when available. Cannabis matrix categories for PT assessments will be established as flower, intermediates, and end products, applied as applicable for required heavy metals testing. (See Appendix A, October 28, 2020; and Proficiency Testing Recommendation Section.)
6. Regulatory updates: The appropriate regulatory authority must make timely regulatory updates to Washington Administrative Code (WAC) for the adoption and implementation of the recommended laboratory quality standards for testing laboratories and for transfer of accreditation authority to Ecology. Laboratory quality standard regulatory updates must be completed two years prior to the mandated accreditation transfer date to Ecology. This will ensure Ecology can build its accreditation rules and guidance to adequately address the most up-to-date laboratory quality standard requirements. Updates to the current Ecology accreditation model will include updates needed for the addition of a cannabis accreditation fee structure and cannabis matrix categories. Earlier implementation of the laboratory quality standards could benefit labs and the current accreditation provider before the transfer of accreditation to Ecology.

   At the time of this publication, the WSLCB is the regulatory agency for laboratory quality standards. The WSLCB and WSDA have proposed joint agency request legislation for the 2022 Session to transfer this authority to the WSDA.

Additionally, the WSLCB WAC 314-55-102 presently contains reference to an "inorganic arsenic"[31] action limit. During the course of the CSTF work, the WSLCB members confirmed that they were actively in rulemaking to remove "inorganic arsenic" and replace it with "total arsenic". As a result of this communication, the Heavy Metals Workgroup focused the development of laboratory quality standards around "total arsenic" methodologies and procedures. The Heavy Metals Workgroup recommendations thus only reflect provisions for total arsenic. The recommendations do not provide for or address the highly specialized requirements needed for accurately testing and reporting inorganic arsenic in cannabis products. This discrepancy could result in a gap for the laboratories and for accreditation if the lab standards are adopted prior to establishing a total arsenic action limit in rule.

Residual solvents recommendations

The Cannabis Science Task Force recommendations for residual solvents are as follows[32]:

1. Appropriate approved testing methods: SW-846 Method 8015D - Nonhalogenated Organics using Gas Chromatography/Flame Ionization Detection (GC/FID) and Method 8260D – Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). Appropriate approved companion preparation methods include: EPA SW-846 Method 3585 – Waste Dilution for Volatile Organics and SW-846 Method 5021A – Volatile Organic Compounds in Various Matrices Using Equilibrium Headspace Analysis (Appendix: A, May 24, 2021, and Appendix B)
    a. When newer EPA method versions of the approved test methods or companion preparation methods are published, the newest version should replace the current method requirement and labs will be required to update their methodology accordingly. When the ICT is established, document control and ultimate revision decisions will be under their authority.
2. Method validation protocols: Method validation protocols are as established within each approved method.
3. Method performance criteria: Performance criteria are as established within each approved method. The following additional performance measures and adaptations must supersede, or be used in conjunction with the minimum method requirements, as appropriate (Appendix A, May 24, 2021).
    a. Laboratories must follow the method-specified quality control (QC) as a minimum. Additional QC criteria at the project level may be set by the regulatory agency and/or data users as long as the minimum QC requirements written in the methods are followed.
    b. Methanol and any other solvent listed in WAC 314-55-102 must not be used in any preparation or analysis procedure.

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[31] Arsenic is found in both inorganic arsenic and organic arsenic forms, which together these are referred to as total arsenic.
[32] The complete anthology of residual solvents recommendations is compiled in Appendix A.

c. Sections describing fuel (gasoline/diesel) specific analysis procedures, such as section 7.4 in Method 8015D, should not be followed.

d. All WSLCB rules for field and product sampling should be followed. Upon receipt of a sample at a lab, the sample treatment should follow the method requirements for preservation and storage that is cited within 8260D, 8015D, or approved companion preparation method.

e. In Method 3585, the term “Appropriate Solvent” must be defined and understood as, “An organic solvent that is capable of accomplishing the dilution of the sample while still able to meet the quality control requirements of this method, the proceeding analytical method, and regulatory requirements, and is NOT a required analyte per WAC 314-55-102.” The selected solvent must be specifically cited in a lab’s standard operating procedure(s).

4. Sampling and homogenization protocols: Sub-sampling and homogenization protocols are as specified in the approved method(s), except as noted for sample receipt and handling protocols (Appendix A, May 24, 2021):

    a. Each sample must individually meet the WSLCB sampling requirements (WAC 315-55-101).

    b. The container must contain a minimum of 2 grams of sample for residual solvents analysis. The total sample amount may contain more product but must allow for the sample size required for residual solvents.

    c. Samples must be submitted in a hard sealable container or syringe. When headspace is encountered, laboratories must either reject the sample, or flag the data as having biased results due to headspace.

    d. Homogenization of residual solvent samples by the lab is prohibited unless necessary due to sample composition. If homogenization is necessary, steps must be taken to minimize evaporative loss.

    e. If any field QC is submitted (e.g., field blanks, trip blanks), the lab must follow the applicable steps in the approved methods for these samples.

5. Proficiency testing (PT): Residual solvent PT must be performed on samples that are matrix matched or “in-matrix” to assess method performance. Matrix categories for PT assessments will be established as flower, intermediates, and end products, applied as applicable for required residual solvent testing. (See Appendix A, October 28, 2020; and Proficiency Testing Recommendation Section.)

6. Regulatory updates: The appropriate regulatory authority must make timely regulatory updates to Washington Administrative Code (WAC) for the adoption and implementation of the recommended laboratory quality standards for testing laboratories and for transfer of accreditation authority to Ecology. Laboratory quality standard regulatory updates must be completed two years prior to the mandated accreditation transfer date to Ecology. This will ensure Ecology can build its accreditation rules and guidance to adequately address the most up-to-date laboratory quality standard requirements. Updates to the current Ecology accreditation model will include updates needed for the addition of a cannabis accreditation fee structure and cannabis matrix categories. Earlier implementation of the

laboratory quality standards could benefit laboratories and the current accreditation provider before the transfer of accreditation to Ecology.

At the time of this publication, the WSLCB is the regulatory agency for laboratory quality standards. The WSLCB and WSDA have proposed joint agency request legislation for the 2022 Session to transfer this authority to the WSDA.

The current list of residual solvents in WSCLB WAC 314-55-102 is used by laboratories as the required analyte testing list. The CSTF recommended including Chemical Abstract Service numbers to each solvent listed to clearly define which solvents and isomers of those solvents need to be tested.

Microbiological recommendations

The initial Cannabis Science Task Force recommendation for microbiological contaminants is as follows:

> Laboratories must utilize molecular methodology to detect the presence of *Salmonella* spp. and pathogenic *Escherichia coli* (*E. coli*) in cannabis and cannabis containing products. (Appendix A, June 11, 2021)

As described earlier in this report, the CSTF will continue to develop recommendations for laboratory quality standards through early 2022. Further recommendations addressing approved methods, validation protocols, and performance specifications, lab sampling and homogenization, and needed regulatory updates will be assembled and submitted to WSLCB to house until the ICT is formed. The ICT should ensure the microbiological recommendations are incorporated into rule to provide policy for the cannabis laboratories to follow and for Ecology to accredit against. The ICT should possess the authority to facilitate, maintain and provide guidance to laboratories on all rule required methods and protocols.

Proficiency Testing Program Recommendations

Participation in proficiency testing (PT) evaluations is required for initial accreditation and ongoing accreditation assessments of competency for all major testing programs, including those supporting environmental, agricultural, and public health regulations. PT evaluations provide an independent demonstration of competence. They help to instill confidence in testing practices for regulatory bodies and consumers.

As a tool for accreditation, results from independently run PT evaluations help auditors to determine that a lab has demonstrated competency to implement testing methodologies and the capability to produce accurate results. It is imperative that laboratories perform PT sample testing on matrix forms that are as similar as possible to the samples that labs test on a daily basis. Suitable matrix-specific PT samples help to ensure technical performance capabilities. They also help auditors to compare the current status of a lab with past performance and help to identify improvement areas across the scope of matrices encountered regularly. Unfortunately, for the cannabis testing industry, cannabis-matrix (THC > 0.3%) PT samples are not available to Washington laboratories due to the federal legal status and prohibition on interstate transport of cannabis and cannabis products. As a result, Washington's cannabis testing labs have not been assessed to the same scientific testing conditions as other regulatory testing industries.[33]

To facilitate the application of vital in-matrix PT, the PT program must be conducted in Washington using cannabis and cannabis product from inside the state. The CSTF PT recommendations were developed around this premise. The recommendations encompass a programmatic approach to establish a framework and strategy to cover all cannabis fields of testing utilizing either a state-run or third-party PT provider scenario, or combination of both.

The CSTF PT recommendations are presented starting with the highest level framework attributes. Subsequent recommendations then proceed into fundamental PT requirements and items for implementing cannabis matrix proficiency testing. The CSTF recommended[34]:

> The need for statute and rule changes to allow a PT provider to exist in state to produce in-matrix PTs. This would allow either a state agency or private sector PT provider to fill this role. (Appendix A, November 20, 2020)

Once those policy barriers are cleared, to be authorized as a PT provider, the CSTF recommended that a PT provider must meet the following minimum requirements to operate in Washington State[35]:

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[33] Other regulatory testing industries are free to order their industry's matrix-specific PT samples from companies operating outside of the state and participate in national PT studies required for their certification or accreditation. Some exceptions occur for some radiochemical and emerging contaminant PT samples.

[34] The following indented content in this section presents the recommendation language with minor edits for clarity. The complete anthology of proficiency testing recommendations is compiled in Appendix A.

[35] The following indented content in this section presents the recommendation language with minor edits for clarity. The complete anthology of proficiency testing recommendations is compiled in Appendix A.

A PT provider must:

- Acquire and maintain all necessary and appropriate licenses, as required in law and rule, to buy, possess, manufacture, transport, and sell cannabis PTs to Washington State cannabis testing laboratories.
- Be compliant in International Organization for Standardization (ISO) / International Electrotechnical Commission (IEC) 17043 and if they also manufacture the reference material, they must also be compliant with ISO/IEC 17034.
- Able to provide a Certified Reference Material or Certificate of Analysis of the standards used to verify PT true value upon request.
- Use an ISO 17025 and/or Ecology's Laboratory Accreditation Unit accredited method if a true value is utilized for pass/fail of the PT, instead of a consensus mean. Lab must be independent from those participating in the PT Study.
- Maintain traceability of material used to generate the PT material up until transfer to the participating lab.
- Manufacture PT samples with target analyte concentrations similar to compliance samples.
- Run primary PTs every six months and be capable of running rapid-return studies if needed.
- Notify the participating labs at least once, 30 days in advance of the opening of the study.
- Notify the participating labs 48 hours prior to the availability of the study samples, and provide information on when and how schedule pick-up by a participating lab or licensed courier service. (Appendix A, December 17, 2020)

Cannabis accreditation categories are needed for PT samples to serve as an effective tool for accreditation and to match Ecology's established accreditation model. The accreditation categories ensure that the scope of everyday cannabis and cannabis products tested are represented in accreditation assessments. Accreditation categories specifically support Ecology's parameter-based accreditations, which is an assessment based on the combination of a specific analyte, performed using a specific analytical method, in a specific matrix. Leveraging off the current WSLCB product categories, the CSTF recommended[36]:

Accreditation of cannabis labs must be in the following matrix categories: Flower, Intermediate Products and End Products.

- Flower: Cannabis spp. plant material. Not altered or extracted.
- Intermediate Products: Cannabis concentrate or cannabis-infused product that must be or are intended to be converted further to an end product.
- End Products: A refined cannabis product that must not or is not intended to receive further processing prior to retail sale. (Appendix A, September 17, 2020)

[36] The following indented content in this section presents the recommendation language with minor edits for clarity. The complete anthology of proficiency testing recommendations is compiled in Appendix A.

For some fields of testing, the need to have cannabis-matrix PTs is crucial. Where robust laboratory quality standards, and quality systems mechanisms for technical assistance and quality assurance are not in place, as exists currently, matrix PT samples are more essential to verify laboratory assessments. At a minimum, there are three fields of testing that must have at least one in-matrix PT for each matrix category (flower, intermediate, and end product, as appropriate) available at the time accreditation transfers to Ecology. The CSTF recommended to[35]:

> Establish which current fields of testing need to have in-matrix PTs available.
>
> 1. In-matrix PTs are critical to assess method performance for:
>   - Potency
>   - Pesticides
>   - Residual solvents
> 2. In-matrix PTs must be available for potency, pesticides, and residual solvents initially.
> 3. The initial fields must be followed sequentially by the less critical: mycotoxins, terpenes, microbial analysis, metals, water activity, moisture content, and then foreign matter. (Appendix A, October 28, 2020)

Earlier availability of cannabis-matrix PT samples would benefit the current certification system to more accurately assess lab testing capabilities and may increase consumer confidence prior to the accreditation authority transfer.

Within each regulatory industry, proficiency testing (PT) evaluations are required at an established frequency that often aligns with accreditation cycles. Additional provisions may address testing where more risk is involved, such as requiring more frequent PT evaluations when testing human health parameters, or less frequent based on a history of successful performance. The CSTF recommended to[37]:

> Establish the minimum frequency and provisions with which cannabis laboratories analyze PT samples to be consistent with Ecology's environmental and drinking water accreditation model, as is outlined in Ecology's Laboratory Accreditation Unit manual[38]. Minimums will apply once the transfer of accreditation to Ecology has been completed. Additional PT evaluations might be required to maintain accreditation based on individual lab performance.
>
> 1. For initial accreditation (laboratories not currently accredited by the WSLCB's current certification provider, the RJ Lee Group):
>   - The most recent set of satisfactory PT study results must be submitted for each chemistry and microbiological parameter. Results must be submitted before Ecology's Laboratory Accreditation Unit will schedule an on-site assessment.

[37] The following indented content in this section presents the recommendation language with minor edits for clarity. The complete anthology of proficiency testing recommendations is compiled in Appendix A.

[38] https://apps.ecology.wa.gov/publications/SummaryPages/1003048.html

- Satisfactory PT study results are also required when a significant method change occurs for a parameter. Results must be submitted before Ecology's Laboratory Accreditation Unit will schedule an on-site assessment.

2. For ongoing accreditation, and for laboratories currently accredited by WSLCB's current certification provider, the RJ Lee Group, upon transfer of accreditation authority to Ecology:
   - A lab must participate in two PT studies for each applicable parameter each accreditation year, except for microbiology parameters where one study per year is required.
   - The Laboratory Accreditation Unit decides the availability of PTs for specific parameters. The laboratory must ensure required PT samples are analyzed and that the results are reported to the Laboratory Accreditation Unit.
   - For chemistry parameters, after an accredited laboratory submits two satisfactory PT sample results and no unsatisfactory results in an accreditation year, the laboratory is required to submit only one satisfactory PT sample result in subsequent accreditation years. This applies as long as there are no intervening unsatisfactory PT sample results. If the laboratory requests updates or changes to its Scope of Accreditation between renewals, processing will include review of all PT results available at that time.
   - For transferring laboratories, at the time of transfer each laboratory must submit to Ecology all PT data from the last two years. This data should contain each required analyte under the Field of Testing model in at least one matrix. This must be done before the Laboratory Accreditation Unit will schedule the on-site assessment. (Appendix A, December 17, 2020)

Finally, the CSTF explored additional roles for the ICT. Activities leading to the development and facilitation of Cannabis-Matrix PT Program must be accomplished independent of and ahead of the transfer of accreditation authority.[39] As a tool for accreditation, it is imperative that the critical matrix cannabis PT samples are available for Ecology's accreditation assessments to begin. Additional PT related technical assistance and maintenance must be ongoing and carried out in a timely manner to serve as a valuable resource for labs and accreditation. Therefore, the CSTF recommended to[40]:

> Outline additional roles the Interagency Cooperative Team (ICT) would fill in the oversight of the in-state PT provider(s).
>
> The ICT should, at a minimum:
>
> - Investigate PT quality issues of third party providers.
> - If the conditions are such that a third party state run contract is necessary, the following recommendations are made:

[39] For PT to remain an impartial assessment tool for accreditation, the development of PT schemes, PT samples, contract facilitation, and ongoing liaison activities with providers must be constructed and conducted independently from accreditation.
[40] The following indented content in this section presents the recommendation language with minor edits for clarity. The complete anthology of proficiency testing recommendations is compiled in Appendix A.

- Serve as the liaison between the labs and PT provider for items such as scheduling, transport, and quality concerns.
- Develop the scope of contract(s) with a third party PT provider.
- Review bids of contract(s) with a third party PT provider to ensure Ecology's Laboratory Accreditation Unit, applicable RCWs/WACs, and CSTF requirements are met. (Appendix A, January 25, 2021)

Recurring Challenges and Topics of Concern

Throughout the Cannabis Science Task Force (CSTF) discussions, topics emerged that were identified as outside of the main scope of the CSTF's goal to deliver standards for laboratory processes. While not considered as laboratory quality standards, these items may impact the current testing practices, potentially undermining the delivery of accurate test results.[41]

Sampling of cannabis and cannabis products

The inadequate condition and insufficient quantity of samples arriving at a laboratory were identified as systemic problems. Each workgroup identified challenges stemming from sampling that placed regular or daily burdens on laboratories, or were anticipated further complicate CSTF recommendations for robust methods and laboratory processes. Cannabis sample and sample receiving issues identified include:

- Evidence of adulteration or treatment of the sample in attempt to prevent sample results that would require a producer or processor to destroy the lot/ batch represented.
- Samples arriving in cartridges or packaging that cannot be opened without compromising the sample.
- Too little sample to perform the required testing methods.
- Only one sample provided which is difficult to abide by divergent sub-sampling conventions needed to generate accurate data across all chemical and microbiological testing fields.
- Inconsistent sample refusal actions implemented by laboratories and subjective interpretation of WSLCB rules.
- No technical assistance/oversight for sample receiving issues.

The CSTF acknowledged these items and the potential negative impact on laboratory testing practices; however, updates to sampling standards and rules fall outside of the scope of the task force. Other states with legalized cannabis testing have begun to more firmly establish requirements and rules to address the association of adequate sampling processes and their integral relationship to the acquisition of accurate lab testing results. If the need for sampling updates develops in Washington, states such as Oregon, Colorado, and California could be studied for how they have instituted sampler training and sampler credentials requirements, and provided standardized sampling procedures, to strengthen sampling as an important precursor to cannabis testing activities. Federal programs with well-established sampling procedures such as the EPA, FDA, and USDA, including new USDA hemp divisions, may also provide valuable resources. The use of scientifically recognized sampling principles and procedures helps to ensure that representative samples are provided to the laboratories. This supports the robust testing protocols used in the labs, and result in more accurate and meaningful data.

[41] Several of these items were also acknowledged in the Cannabis Laboratory Accreditation Recommendations report in early 2019 and subsequently spurred discussion and recommendations in the 2020 Legislative report.

Cannabis blank matrix and reference materials

The absence of adequate and appropriate sources of cannabis blank matrix and reference materials were prominent discussion topics in several workgroups, most notably in the pesticides and PT workgroups.

For many test methods, there is a basic assumption that matrix-matched blanks and matrix-specific reference materials are available to initially set up the method in a laboratory. Matrix blank and reference materials are needed to calibrate instruments as well as use in initial method performance and validation assessments to demonstrate that the procedure is suitable for its intended purposes. Matching the matrix of the initial performance evaluations to that of what will be tested once the method is implemented is a crucial piece of the demonstration. Matrix blanks and reference materials are also expected to be used during the daily operation of methods and serve as vital routine performance and quality control samples. However, for Washington State cannabis testing laboratories, both federal and state regulations on possessing and transporting cannabis materials containing THC greater than 0.3% hinder the availability of these materials and the subsequent implementation of these fundamental quality control pieces.

Without a reliable source of matrix blank, a burdensome pathway is created that requires laboratories to either collect leftover “blank” sample matrix material, determined to be free of the contaminants being tested for, or purchase additional cannabis products from WSLCB-licensed cannabis retail stores at the limited personal-use quantity of one ounce. Further, cannabis-matrix reference materials, or matrix materials that have been certified as ‘controls’ or measurement standards, simply do not exist. As with cannabis matrix PT samples, there is no current ISO 17034 qualified reference material provider producing these materials within the state and no ability for labs to access materials from sources outside of this state. If barriers for obtaining, possessing and transporting cannabis matrix materials continue exist as they do today, these hindrances may stall the ability of a lab to appropriately implement established laboratory quality standards, including required methods, and may result in the inability for Ecology to grant accreditations.

Barriers and challenges to matrix blank and reference material acquisition was also highlighted in the first Cannabis Science Task Force report in 2019.[42] Captured also as a side issue in that report, these materials were originally identified as crucial for the appropriate implementation of the Cannabis Science Task Force recommendation for the use of the adapted USDA protocols for pesticides.

[42] https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html

Conclusion

The cannabis industry has evolved more rapidly than the science needed to support it. Laboratory science needs to be conducted with high standards to ensure that cannabis products meet regulations to protect consumers. For many of the required fields of testing, testing methods have largely been generated by private laboratories with various kinds of equipment and different levels of expertise. Instances of inconsistent results among laboratories reduced consumer confidence and led the Legislature to seek options for accrediting laboratories to ensure credible and consistent results and improving consumer protections.

In 2019, the Legislature identified the Washington State Department of Ecology (Ecology), with its expertise in accrediting environmental and drinking water laboratories, as the appropriate laboratory accreditation body for cannabis. However, in order to apply Ecology's laboratory accreditation program, the Legislature also recognized that standardized testing methods and improved proficiency testing protocols were needed. To do that, the Cannabis Science Task Force (CSTF) was formed and worked for the past two years to create scientific recommendations needed to standardize the laboratory testing practices and to transfer accreditation to Ecology.

The CSTF work was divided into different fields of testing that cumulated in two legislative reports. The first report addressed laboratory science to test for pesticides in plants and products. This second report addresses other fields of testing (potency, heavy metals, and residual solvents) as well as proficiency testing that must be applied to all fields. Clarity on details achieved through the CSTF's work underscored unforeseen needs to (1) create an on-going regulatory oversight body with scientific expertise and (2) develop a proficiency testing program to use cannabis (not hemp) materials for blind proficiency test samples. Resources and subject matter expertise were identified as barriers for implementing the oversight body. Interstate commerce and licensing were noted barriers in developing the proficiency testing program. The oversight body is needed not only to implement these improved lab quality standards, but also serve as ongoing technical experts to manage the evolution of the science in this industry. Additionally, a cannabis-based proficiency testing program is required for Ecology's accreditation program to operate and provide a viable pathway for cannabis laboratory accreditation. The CSTF has identified these two gaps as needing resolution, which in combination with the improved lab quality standards described here, will achieve the desired results of improved consumer protections.

Definitions and Acronyms

Accreditation (WAC 173-50 definition) – The formal recognition by the department [Ecology] that an environmental laboratory is capable of producing accurate and defensible analytical data. This recognition is signified by the issuance of a written certificate, accompanied by a scope of accreditation indicating the parameters for which the laboratory is accredited. The term "accredit" as used in this chapter is intended to have the same meaning as the term "certify" as used in RCW 43.21A.230.

Accreditation categories – Divisions necessary for granting accreditation by parameter (matrix, analyte, and analytical method). Current accreditation (matrix) categories are *Drinking Water*, *Non-Potable Water*, *Solids and Chemical Materials*, and *Air and Emissions*.

Accreditation standards (as used within this report) – Established criteria that describe the accreditation evaluation process to ensure accredited laboratories have the demonstrated capability to provide accurate, defensible data. Accreditation standards include descriptions of authority (i.e., granting, denying, suspending, and revoking accreditation), accreditation certification cycle length (e.g., 1-year period), on-site audit frequencies, application process, fee structure, and other procedural specifics of the accreditation process. More specifically, the accreditation standard may identify critical items (e.g., appropriate implementation and use of methods and standard operating procedures, use of quality control samples, and passing proficiency testing sample results) that will be assessed or evaluated as a part of the accreditation process.

Analytical method – A procedure consisting of several laboratory procedures, which when completed, produces a quantitative and/or qualitative result for the tested substance.

Analytical data – The qualitative or quantitative results from a chemical, physical, microbiological, toxicological, radiochemical, or other scientific determination.

Blank matrix – A matrix that does not produce an analytical response by the analytical method under investigation for the analytes(s) of interest (USDA, 2015).

Cannabis (as used within this report) – Cannabis spp. plant or cannabis materials containing > 0.3% delta-9 tetrahydrocannabinol.

Cannabis Accreditation Categories (as recommended in this report) – An extension of the definition of *Accreditation Categories* to incorporate cannabis matrices:

- Flower: Cannabis spp. plant material. Not altered or extracted.
- Intermediate Products: Cannabis concentrate or cannabis infused product that must be or are intended to be converted further to an end product.
- End Products: A refined cannabis product that must not or is not intended to receive further processing prior to retail sale.

Cannabis matrix (as used within this report) – An extension of the definition of *Matrix* to include cannabis plant (flower) material, cannabis intermediates (such as concentrates and cannabis infused products), and refined cannabis end products (such as edible products and lotions).

Certified reference material – A reference material one or more of whose property values are certified by technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certified body (NELAC, 2003).

Client (as used within this report) – A regulatory agency identified entity housing personnel with authority and expertise to adopt and establish rule (or guidance) for laboratory quality standards based on sound science practices. The entity additionally serves to establish, maintain, and provide technical assistance for adopted laboratory quality standards.

Collaborative study – see *Inter-laboratory study.*

In-lab sampling or sub-sampling is a procedure by which a small, representative sample is taken from a larger sample.

Inter-laboratory study – Organization, performance and evaluation of measurements or tests on the same or similar items within two or more laboratories in accordance with predetermined conditions. (ISO/IEC, 2017)

Intra-laboratory study – Organization, performance and evaluation of measurements or tests on the same or similar items within the same laboratory in accordance with predetermined conditions. (ISO/IEC, 2017)

Laboratory quality standards (as used within this report) – Established criteria designed to produce accurate and reproducible data. Deliberate and intentionally designed laboratory quality standards ensure that established product standards can be met. In broad terms, laboratory quality standards are defined methods, method validation protocols, and performance criteria (e.g., use of quality control samples and their tolerance limits). These provide laboratories standardized requirements to follow, and also give accreditation providers critical elements to assess during the accreditation process.

Matrix – The substance from which a sample is collected, such as groundwater, ambient water, wastewater, air, solid, semisolid (such as tissue), or chemical compounds (such as oil). (Ecology, 2010) See also *Cannabis matrix***.**

Matrix blank – A substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. The matrix blank is used to determine the absence of significant interference due to matrix, reagents and equipment used in the analysis. (FDA, 2019)

Method validation – The process of demonstrating or confirming that a method is suitable for its intended purpose. Validation criteria include demonstrating performance characteristics such as accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, ruggedness and robustness. (FDA, 2019)

Parameter – The combination of one or more analytes determined by a specific analytical method. (Ecology, 2010)

Performance-based methods – Approach conveys "what" needs to be accomplished, but not prescriptively "how" to do it. It is a measurement system based upon established performance criteria for accuracy and precision with use of analytical test methods. Under this measurement system, laboratories must demonstrate that a particular analytical test method is acceptable for demonstrating compliance. Performance-based method criteria may be published in regulations, technical guidance documents, permits, work plans, or enforcement orders.

Performance criteria (or measures) – Defined, measurable performance characteristics of an analytical method or process-specific requirements for accuracy, precision, recovery, specificity (selectivity), sensitivity (limits of detection), inclusivity, exclusivity, linearity, range, and scope of application. Criteria may also be set by defining process (i.e., method validation protocols).

Proficiency testing (PT**)** – A means of evaluating a laboratory's performance under controlled condition relative to a given set of criteria through analysis of unknown samples provide by an external source (NELAC, 2003).

Proficiency testing sample (PT sample) – The sample provided to a laboratory for the purpose of demonstrating that the laboratory can successfully analyze the sample within acceptance limits specified in the regulations. The qualitative and/or quantitative composition of the reference material is unknown to the laboratory at the time of the analysis (EPA, 2005).

Product standards (as used within this report) – Established regulatory requirements that products or materials that are produced for consumers must meet. Compliant products under these standards are asserted to be safe, free from contaminants, and produced to a specified composition or dosage requirement. Current cannabis standards include potency levels, pesticides action limits, mycotoxin limits, packaging requirements, and others.

Quality assurance (QA) – An integrated system of management activities involving planning, implementation, documentation, assessment, reporting, and quality improvement to ensure that a process, item, or service is of the type and quality needed and expected by the client (EPA, 2001).

Quality systems – A structured and documented system describing the policies, objectives, principles, organizational authority, responsibilities, accountability, and implementation plan of an organization for ensuring the quality in its work processes, products, items, and services. The quality system provides the framework for planning, implementing, and assessing work performed by the organization and for carrying out required QA and QC. (EPA, 2002)

Reference Materials – Material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process.

Sample – Representative portion of material taken from a larger quantity of homogenate for the purpose of examination or analysis which can be used for judging the quality of a larger quantity.

Standard operating procedure (SOP) – A written document that details the method for an operation, analysis, or action with thoroughly prescribed techniques and steps, and that is officially approved as the method for performing certain routine or repetitive tasks (EPA, 2001).

SW-846 – Test Methods for Evaluating Solid Wastes: Physical/Chemical Methods Compendium. See The SW-846 Compendium | US EPA

Validated methods – The methods that have undergone validation.

Validation (method) – The process of demonstrating or confirming the performance characteristics through assessments of data quality indicators for a method of analysis.

Acronyms

AOAC	Association of Official Analytical Chemists
CSTF	Cannabis Science Task Force
DOH	Department of Health
Ecology	Washington State Department of Ecology
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
ICT	Interagency Cooperative Team
NYS	New York State
PDP	Pesticide Data Program
PT	Proficiency testing
RCW	Revised Code of Washington
SOP	Standard operating procedure
SW-846	(see Definitions above)
USDA	U.S. Department of Agriculture
WAC	Washington Administrative Code
WSDA	Washington State Department of Agriculture
WSLCB	Washington State Liquor and Cannabis Board

References

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Sekerak, Sara, 2020. Cannabis Science Task Force Recommendations: Laboratory Quality Standards for Pesticide in Cannabis Plants and Products. Washington State Department of Ecology https://apps.ecology.wa.gov/publications/SummaryPages/2003005.html

Appendix A. Motions Recommended by the Cannabis Science Task Force

For each of the motions below, and as outlined in the Charter:

- The Chair recognized a member who makes a motion
- The Chair recognized another member who seconds the motion
- The motion was restated to the assembly and for the record
- The members debated the motion, as necessary
- The Chair asks for the affirmative votes and then the negative votes
- The Chair announces the result of the voting

The member who makes the motion and the member who seconds the motion are each noted below the recorded motion. A motion passed when a simple majority voted affirmatively; negative votes are noted as they occurred. Motions providing recommendations for the second RCW 43.21A.735 mandated report are presented in chronological order, by workgroup. The following motions exhibit the unedited language approved by the Task Force.

January 30, 2020 - Proficiency Testing Workgroup

MOTION #1: Motion to establish the requirement that cannabis proficiency testing providers must be compliant with ISO/IEC 17043, and if they also manufacture the reference material, they must be compliant with ISO 17034.

Motion: Amber Wise
Second: Nick Mosely
Vote Result: Pass

July 29, 2020 - Potency Workgroup

MOTION #1: The potency test will be an “as is” test.

Motion: Brad White
Second: Amber Wise
Vote Result: Pass

September 17, 2020 - Proficiency Testing Workgroup

MOTION #1: Accreditation categories for cannabis

Accreditation of cannabis labs shall be in the following matrix categories: Flower, Intermediate Products and End Products.

- Flower: Cannabis sp. plant material. Not altered or extracted. Intermediate
- Products: Cannabis concentrate or cannabis infused product that must be or are intended to be converted further to an end product.

- End Products: A refined Cannabis product that must not or is not intended to receive further processing prior to retail sale.

Motion: Jessica Archer
Second: Amber Wise
Vote Result: Pass

October 28, 2020 - Proficiency Testing Workgroup

MOTION #1: Required Matrix-Matching Priority for PTs

This motion would establish which current fields of testing need to have in-matrix PTs available. In-matrix PTs are critical to assess method performance for potency, pesticides and residual solvents. In- matrix PTs must be available for potency, pesticides, and residual solvents initially. These PTs will be followed sequentially by the less critical mycotoxins, terpenes, microbial analysis, metals, water activity, moisture content, and then foreign matter.

Motion: Amber Wise
Second: Jessica Archer
Vote Result: Pass

October 28, 2020 - Heavy Metals Workgroup

MOTION #1: We will accept the following approved SW-846 compendium methods with specific cannabis changes where indicated:

- **Method 6020B: Inductively Coupled Plasma-Mass Spectrometry.**
- **Method 6010D: Inductively Coupled Plasma-Optical Emission Spectrometry.**

This motion is intended to drive the metals testing of cannabis and cannabis related products into a narrower field of allowable method platforms following an approach that allows some flexibility which incorporates performance-based criteria options.

Motion: Jessica Archer
Second: Nick Mosely
Vote Result: Pass
Negative Vote: Jeff Doughty – stated that the labs should be able to use whatever they can prove can perform the testing.

November 20, 2020 - Potency Workgroup

MOTION: Adopt The NYS DOH MML-300 And NYS DOH MML 301 as reference methods for potency testing as a basis. Adaptations will be provided in future motions.

This motion will use the New York Department of Health Methods for potency sample preparation and analysis as a basis. Future motions will provide adaptations to the method needed for Washington State.

Motion: Jessica Archer
Second: Nick Mosely
Vote Result: Pass

November 20, 2020 - Proficiency Testing Workgroup

MOTION #1: Allowing for an In-State PT Provider

This motion recognizes the need for statute and rule changes to allow a PT provider to exist in state to produce in-matrix PTs. This would allow either a state agency or private sector PT provider to fill this role.

Motion: Amber Wise
Second: Nick Mosely
Vote Result: Pass

December 17, 2020 - Proficiency Testing Workgroup

MOTION #1: Minimum requirements of a PT Provider

This motion outlines the minimum requirements a PT provider needs to meet in order to operate in Washington State.

A PT Provider must:

- Acquire and maintain all necessary and appropriate licenses, as required in law and rule, to buy, possess, manufacture, transport, and sell cannabis PTs to Washington State cannabis testing laboratories.
- Be compliant in ISO/IEC 17043 and if they also manufacture the reference material, they must also be compliant with ISO/IEC 17034 (as per 1/30/2020 Proficiency Testing Workgroup motion)
- Able to provide a Certified Reference Material or Certificate of Analysis of the standards used to verify PT true value upon request.
- Use an ISO 17025 and/or Ecology's Laboratory Accreditation Unit accredited method if a true value is utilized for pass/fail of the PT, instead of a consensus mean. Lab must be independent from those participating in the PT Study.
- Maintain traceability of material used to generate the PT material up until transfer to the participating lab.
- Manufacture PT samples with target analyte concentrations similar to compliance samples.
- Run primary PTs every six months and be capable of running rapid-return studies if needed.
- Notify the participating labs at least once, 30 days in advance of the opening of the study.
- Notify the participating labs 48 hours prior to the availability of the study samples, and provide information on when and how schedule pick-up by a participating lab or licensed courier service.

Motion: Amber Wise
Second: Kendra Hodgson
Vote Result: Pass

MOTION #2: Minimum required PT frequency

This motion would establish the minimum frequency with which cannabis laboratories analyze PTs, consistent with Ecology’s Lab Accreditation Unit manual. More might be required to

maintain accreditation based on individual lab performance. Applies once the transfer of accreditation to LAU has been completed.

For initial accreditation of a lab not previously accredited by RJ Lee/WSLCB: The most recent set of satisfactory PT study results must be submitted for each chemistry and micro parameter. This must be done before the Lab Accreditation Unit will schedule the on-site assessment. This is also required when a significant method change occurs for a parameter.

For ongoing accreditation: The lab must participate in two PT studies for each applicable parameter each accreditation year, except for microbiology parameters where one study per year is required. The Lab Accreditation Unit decides the availability of PTs for specific parameters. The lab must ensure required PT samples are analyzed and that the results are reported to the Lab Accreditation Unit. For chemistry parameters, after an accredited lab submits two satisfactory PT sample results and no unsatisfactory results in an accreditation year, the lab is required to submit only one satisfactory PT sample result in subsequent accreditation years. This applies as long as there are no intervening unsatisfactory PT sample results. If the lab requests updates or changes to its Scope of Accreditation between renewals, processing will include review of all PT results available at that time.

Motion: Amber Wise
Second: Nick Mosely
Vote Result: Pass

January 25, 2021 - Proficiency Testing Workgroup

Motion #1: PT Roles for the Interagency Cooperative Team (ICT)

This motion would outline additional roles the Interagency Cooperative Team (ICT) would fill in oversight of the in-state PT provider(s).

The ICT should, at a minimum:

- Investigate PT quality issues of 3rd party providers
- If the conditions are such that a 3rd party state run contract are necessary, the following recommendations are made.
  - Serve as the liaison between the labs and PT provider for items such as scheduling, transport, and quality concerns
  - Develop the scope of any necessary contract(s) with a 3rd party PT provider
  - Review bids of any necessary contract(s) with a 3rd party PT provider to ensure LAU[43], applicable RCWs/WACs, and CSTF requirements are met

Motion: Amber Wise
Second: Jeff Doughty
Vote Result: Pass

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[43] LAU: [Ecology's] Laboratory Accreditation Unit

MOTION #2: PT records needed for labs transferring from RJ LEE/WSLCB to ECY's Lab Accreditation Unit

This motion would outline what minimum PT records are necessary for labs transferring accreditation from RJ Lee/WSLCB to ECY's Lab Accreditation Unit. At a minimum, all PT data in the last 2 years will be submitted by the labs. This data should contain each required analyte under the Field of Testing model in at least one matrix. This must be done before the Lab Accreditation Unit will schedule the on-site assessment.

Motion: Kendra Hodgson
Second: Amber Wise
Vote Result: Pass

February 22, 2021 - Heavy Metals Workgroup

MOTION AMENDMENT: Required use of the most current SW-846 Compendium Methods for metals testing in cannabis and cannabis related products.

This motion amends the Steering Committee approved Heavy Metals Workgroup motion from October 28, 2020 for the initial SW- 846 methods requirement for testing metals in cannabis and cannabis related products.

Amendment: When newer EPA method versions of SW-846 Method 6020B and SW-846 Method 6010D are published, the newest version shall replace the current method requirement and labs will be required to update their methodology accordingly. If and when the ICT is established, document control and ultimate revision decisions will be under their authority.

Motion: Amber Wise
Second: Kendra Hodgson
Vote Result: Pass

MOTION #2: Requirement of SW-846 Companion Preparation Methods for metals testing in cannabis and cannabis related products.

This motion requires that the preparation method(s) for metals testing in cannabis and cannabis products be one of the following SW-846 Companion Methods: EPA 3031, 3050B, or 3052 with the understanding that any subsequent versions which are published will replace the current method and labs will be required to update their methodology accordingly.

Motion: Amber Wise
Second: Kendra Hodgson
Vote Result: Pass

March 23, 2021 - Heavy Metals Workgroup

MOTION: Metals method adaptations

This motion adopts the following adaptations to the SW-846 Methods 6020B and 6010D, and any subsequent newer versions, required for use for testing cannabis and cannabis products:

1. Ultra high-purity or equivalent acids must be used in the preparation of standards and for sample processing. Redistilled acids are recommended because of the high sensitivity of ICP-MS. It is recommended that the final nitric acid concentration used is between 2-5% (v/v) to minimize damage to the ICP/MS interface and to minimize isobaric molecular-ion interferences with the analytes. Other acid concentrations are acceptable if they are documented in the lab's internal SOP and all performance criteria are met.
2. All analytical standards and solutions are required to be NIST Traceable or equivalent.
3. It is HIGHLY recommended to use CRM and/or SRM materials when available for method development and trouble shooting and for optional additional quality control (QC).
4. It is recommended that the lab follow the QC guidance in the EPA SW-846 methods; however, changes to QC criteria at the project level may be set by the regulatory agency and/or data users as long as the minimum QC requirements written in the methods are followed. More QC is allowed, but less is not.
5. Instruments must be calibrated using a minimum of a four-point curve (no blanks can be used as a point). The correlation determines (r^2) should be ≥ 0.990 or the correlation coefficient (r) should be ≥ 0.995. Use Linear Regression with 1/x or no weight. Forcing the curve through zero is not allowed.

Motion: Amber Wise
Second: Jeff Doughty
Vote Result: Pass

April 29, 2021 – Task Force Steering Committee Motion

MOTION: Redefining Potency Workgroup objectives and scope

This motion defines the current guiding principles, objectives, and scope for addressing the laboratory quality standard recommendations and future pathway deliverables.

Principles

Reduce risk and provide for better consumer protections

Provide for consistency and accuracy in analysis and accreditation

Objectives

Recommend laboratory quality standards for Potency in marijuana products in accordance with RCW 43.21A.735.

(a) Appropriate approved testing methods;
(b) Method validation protocols;
(c) Method performance criteria;
(d) Sampling* and homogenization protocols;
(e) Proficiency testing; and
(f) Regulatory updates related to (a) through (e) of this subsection, by which agencies, and the timing of these updates.

To the fullest extent possible, the task force must consult with other jurisdictions that have established, or are establishing, marijuana product testing programs. *Sampling refers to within laboratory, after the product has been received.

- Establish standardized methods and processes for cannabis testing laboratories to follow and accreditation to accredit to.
- Provide for a pathway to address new regulated parameters (matrix and analyte) not covered by standardized methods.

Scope

- Regulatory required analytes designated in WAC 314-55-102 Potency analysis: THCA, THC, total THC, CBDA, CBD, total CBD, and further defined by RCW 69.50.101(uu) "THC concentration".
- Product matrices not covered in adopted methods may be addressed via future pathways outlined by the Task Force, as time allows. The ICT will assume the role in finalizing the future pathways as described below.
- Non-regulatory components may be addressed by the ICT in the future but are not the scope of the Task Force /workgroup. Future components may include adding analytes, or minor changes to optimize performance, such as changes to columns, but will not include the removal of any method quality control (QC) requirements/samples.

Deliverables

Deliverable One:

Adoption of NY MML-300, NY MML-301, and Task Force Summary of Potency Adaptations for Washington as "Potency Standardized Methods".

Deliverable Two:

The Task Force begins outlining the future pathway for introducing flexibility to "Potency Standardized Methods", beginning with minor matrices/products that are not included in the NY MML-300 and NY MML-301 methods. The outline for this pathway will be included in the final Task Force Legislative report to provide guidance for, and resolution by, the ICT.

The pathway outline shall incorporate the following key attributes for making future modifications to the "Potency Standardized Methods":

- Utilizes skilled cannabis laboratories to advance science in an inter-laboratory study design.
- Includes participation by a state-run laboratory.
- Initially addresses only matrices/products not addressed by NY Method(s).
- Starts only after successful implementation of MML-300/ MML-301, with WA-specific adaptations.
- Consists of ICT-coordinated modifications to MML-300/ MML-301.
- Cannabis laboratories must maintain accreditation and passing PTs of MML-300/ MML-301, with WA-specific adaptations, while performing modification studies.
- All laboratories validate/verify modification by:
  - Workgroup Task: Begin to outline basic validation/verification objectives and process for ICT to resolve/finalize (submitted in a future motion).

- ICT evaluates, compares data, and designates as an official approved method modification available to all laboratories

April 29, 2021 - Residual Solvents Workgroup

MOTION #1: Needed clarifications to the required solvent list from WAC 314-55-102

This motion would outline (minimum) required changes needed to the solvent list in WAC 314-55-102 to ensure consistency and comparability across the cannabis labs in Washington State.

1. Chemical Abstract Service (CAS) numbers for each analyte must be included in the WAC 314-55-102.
2. Any required analyte that has an isomer must identify each required individual isomer by CAS number.

Motion: Amber Wise
Second: Jeff Doughty
Vote Result: Pass

MOTION #3: Recommended clarifications to the required solvent list from WAC 314-55-102

This motion would outline recommended changes needed to the solvent list in WAC 314-55-102.

1. "Heptanes" should be changed to simply "Heptane".
2. Cyclohexane should be removed from the list.
3. Ethanol should be added to the list.

Motion: Kendra Hodgson
Second: Amber Wise
Vote Result: Pass

April 29, 2021 - Potency Workgroup

MOTION #4: Consensus adaptations

This motion would adopt the listed consensus adaptations:

Method NYS DOH MML-301 Revision 6

1. Change references from New York State (NYS) to Washington (WA). Change all references pointing to NYS rule and law to and point to WA rule and law instead.
2. Change references to "Medical Marijuana" to "Marijuana and Marijuana products". Also changes the references in the method to abbreviation. The NY method uses "MM" for medical marijuana.
3. Change references for "Registered Organization (RO)" to "licensed producer or processor".
4. Strike section 2.2.
5. Section 4.2 remove reference to yellow binder. Strike "are located within the laboratory in labeled, yellow binders."
6. Section 7.3.1 add "or other matrix that does not contain cannabinoids".

7. Section 8.3.4 Add "scale volume as necessary".
8. Strike sections 11, 12, - LCB and Ecology rules apply not NY.

Method NYS DOH MML-300 Revision 6

1. Change references from New York State (NYS) to Washington (WA). Change all references pointing to NYS rule and law and to point to WA rule and law instead.
2. Change references to "Medical Marijuana" to "Marijuana and Marijuana products". Also changes the references in the method to abbreviation. The NY method uses "MM" for medical marijuana.
3. Change references for "Registered Organization (RO)" to "licensed producer or processor".
4. Strike section 2.2.
5. Section 5.2.1 and 5.2.2 delete after the first sentence of each point and add "According to lab SOP".
6. Section 5.6 Add. "If standard not available in final solvent, the other solvent may be used."
7. Section 8.1.3 Remove second sentence. Add, "Syringe or positive pressure pipet must be traceable to NIST. Mechanical syringes, if used, must be verified daily, calibrated once a year or more frequently if syringe manual indicates."
8. Add Section 8.8.3. "Standards may be stored at -20°c stored for two months or less. Standards may be stored for up to one year at -80° C."
9. Remove section 9.1.1. LCB has rules for shipping cannabis.
10. Remove 9.2.3.
11. Section 9.4.2.5.1 and 9.4 Change "190 to 800 nm" to "190 to 400 nm".
12. Section 11.9.1 Add. Follow PGF factor (Equation 3) unless your instrument software does not support it. In that case, you may calculate it manually or use TF < 2.0 as defined in EPA method 8270E Section 11.3.1.3.
13. Section 13.4 Replace whole section with "Follow LCB requirements".
14. Remove Section 16. Follow Ecology rules for waste disposal.

Motion: Amber Wise
Second: Kendra Hodgson
Vote Result: Pass

May 24, 2021 - Residual Solvents Workgroup

MOTION #1: Approved analytical methods for residual solvents.

The following SW-846 Compendium Analytical Methods have been approved for analysis of Residual Solvents with specific Cannabis method changes where indicated— Method 8015D Nonhalogenated Organics using GC/FID and Method 8260D Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry

This motion is intended to drive the Residual Solvents testing of cannabis related products into a narrower field of allowable method platforms following an approach that allows some flexibility, which incorporates performance-based criteria options. Upcoming motions will include a Summary of Adaptations for the approved methods.

When newer EPA method versions of SW-846 Method 8015D and SW- 846 Method 8260D are published, the newest version shall replace the current method requirement and labs will be required to update their methodology accordingly. When the ICT is established document control and ultimate revision decisions will be under their authority.

Motion: Jessica Archer
Second: Nick Mosely
Vote Result: Pass

MOTION #2: Consensus residual solvents analytical method adaptations.

This motion adopts the following consensus adaptations to the SW- 846 Methods 8015D and 8260D, and any approved subsequent method revisions, required for use for testing cannabis products for the required solvents (WAC 314-55-102):

1. For both methods, the labs shall follow the QC guidance in the EPA SW-846 methods as a minimum; however, changes to QC criteria at the project level may be set by the regulatory agency and/or data users as long as the minimum QC requirements written in the methods are followed. More QC is allowed, but less is not.
2. Labs shall use only companion preparation methods approved for regulatory use on cannabis products.
3. Methanol and any other solvent listed in WAC 314-55-102 must not be used in any preparation or analysis procedure.
4. Labs shall not follow any sections describing fuel (gasoline/diesel) specific analysis procedures, such as section 7.4 in 8015D.
5. Labs must follow all WSLCB rule for field/product sampling. Upon receipt at the lab, the sample treatment should follow the method requirements for preservation and storage that is cited within 8260D, 8015D, or approved companion preparation method

Motion: Amber Wise
Second: Jeff Doughty
Vote Result: Pass

MOTION #3: Approved preparation methods for residual solvents.

The following SW-846 compendium preparation methods have been approved for analysis of Residual Solvents with specific Cannabis method changes where indicated — METHOD 3585 WASTE DILUTION FOR VOLATILE ORGANICS and METHOD 5021A VOLATILE ORGANIC COMPOUNDS IN VARIOUS SAMPLE MATRICES USING EQUILIBRIUM HEADSPACE ANALYSIS.

This motion is intended to drive the Residual Solvents testing of cannabis related products into a narrower field of allowable method platforms following an approach that allows some flexibility, which incorporates performance-based criteria options. Upcoming motions will include a Summary of Adaptations for the approved methods.

When newer EPA method versions of SW-846 Method 3585 and SW- 846 Method 5021A are published, the newest version shall replace the current method requirement and labs will be required to update their methodology accordingly. If, and when the ICT is established document control and ultimate revision decisions will be under their authority.

Motion: Amber Wise
Second: Jeff Doughty
Vote Result: Pass

MOTION #4: Residual solvents preparation method adaptations.

This motion adopts the following consensus adaptations to the SW- 846 Methods 3585 and 5021A, and any approved subsequent method revisions, required for use for testing cannabis products for the required solvents (WAC 314-55-102):

1. For both methods, labs shall follow the QC guidance in the EPA SW-846 methods as a minimum; however, changes to QC criteria at the project level may be set by the regulatory agency and/or data users as long as the minimum QC requirements written in the methods are followed. More QC is allowed, but less is not.
2. In method 3585, Appropriate Solvent is defined as, "An organic solvent that is capable of accomplishing the dilution of the sample while still able to meet the quality control requirements of this method, the proceeding analytical method, and regulatory requirements and is NOT a required analyte per WAC 314-55-102." This solvent must be specifically cited in the lab's SOP.
3. Labs must follow all WSLCB rule for field/product sampling. Upon receipt at the lab, the sample treatment should follow the method requirements for preservation and storage that is cited within 8260D, 8015D, or approved companion preparation method.

Motion: Jeff Doughty
Second: Kendra Hodgson
Vote Result: Pass

May 24, 2021 - Potency Workgroup

MOTION #1: Guidelines on new potency methods and modifications to potency methods.

Procedure for the adoption of a new method or to modify an existing method:

1. Step 1: A lab that wants to initiate a change, the "sponsor lab", will create and document the new method or modification.
2. Step 2: The sponsor lab shall validate the method according to U.S. Food and Drug Administration (FDA) document "Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products", Level 4. The ICT will need to review the guidelines and make any needed adaptations.
3. Step 3: A collaborative study would then be conducted, using the AOAC Collaborative Study procedure as described in the FDA document for a "level 4" validation. The ICT would need to review the AOAC procedures and make appropriate adaptations. The ICT would need to adapt the procedure prior to use.
4. Step 4: A report would be created of the validation and study and sent to the ICT.
5. Step 5: The ICT would review the report and accept, reject, or request additional data and/or explanation, clarifications. As part of the review the ICT will review data that compares the results of the new method to previously approved methods (at the start this

will just be the NY method but as new methods are approved they will be include) to ensure that the new method has similar accuracy, bias, and measurement uncertainty.

6. Step 6: If approved, the new method or modification will be public and available to all labs to use.

In the event that the sponsor lab cannot obtain the number of labs needed for the AOAC guidelines (8 or 10) then the lab may contact the ICT and ask to be allowed to run the study with fewer labs. The ICT will evaluate the risk both of the lower method quality and the any risks of not validating the method. The ICT may approve the study to be done with fewer labs.

Motion: Jeff Doughty
Second: Kendra Hodgson
Vote Result: Pass

MOTION #2 Regulatory ability to perform collaborative studies

To perform a collaborative study, a lab or a PT provider must create and ship samples to laboratories for testing. This may involve direct payment or payment in kind (supplies, columns, or other items). A sponsor lab might wish to hire a PT provider to create the samples. The industry, potentially non-labs, may wish to commission a lab to create and sponsor a method. A lab may wish to specialize in method development and just do that and not test potency otherwise. Any changes to RCW, WAC or policy needed to allow this activity should be done.

Motion: Kendra Hodgson
Second: Nick Mosely
Vote Result: Pass

MOTION #3 Collaboration study participation

When a sponsor lab plans a collaborative study, all labs that are authorized to preform potency testing must be asked if they wish to participate in the study and if the lab does want to participate, they must be included in the study.

Motion: Jeff Doughty
Second: Amber Wise
Vote Result: Pass

MOTION #4 State lab participation

State Lab(s) testing for potency should be included in the list of labs invited to participate in collaborative studies and be allowed to be a sponsor laboratory. Any approved ISO compliant PT providers should be allowed to provide samples for validation studies and collaborative studies.

Motion: Jeff Doughty
Second: Kendra Hodgson
Vote Result: Pass

June 11, 2021 - Residual Solvents Workgroup

MOTION #1: Guidance on residual solvents sample receipt/handling

This motion outlines minimum acceptance requirements for residual solvent samples in order to increase consistency and comparability in Residual Solvents testing. Laboratories must reject samples if the following requirements are not met.

1. Each sample must individually meet the WSLCB sampling requirements (WAC 315-55-101).
2. The container must contain a minimum of 2 g of sample for residual solvents analysis. The total sample amount may contain more product but must allow for the sample size required for residual solvents.
3. Samples must be submitted in a hard sealable container or syringe. When headspace is encountered, laboratories must either reject the sample, or flag the data as having biased results due to headspace.
4. Homogenization of residual solvent samples by the lab is prohibited unless necessary due to sample composition. If homogenization is necessary, steps must be taken to minimize evaporative loss.
5. If any field QC is submitted (e.g., field blanks, trip blanks) the lab must follow the applicable steps in the approved methods for these samples

Motion: Amber Wise
Second: Jessica Archer
Vote Result: Pass

June 11, 2021 - Microbiological Workgroup

MOTION #1: Molecular screening methods for Salmonella and pathogenic Escherichia coli

This motion would require laboratories to utilize molecular methodology to detect the presence of *Salmonella* spp. and pathogenic *Escherichia coli* (*E. coli*) in cannabis and cannabis containing products.

Motion: Kendra Hodgson
Second: Amber Wise
Vote Result: Pass

Appendix B. Methods Recommended by the Cannabis Science Task Force

Potency

- NYS DOH MML-301, Revision 6: Medical Marijuana Sample Preparation Protocols For Potency Analysis
- NYS DOH MML-300, Revision 6: Measurement Of Phytocannabinoids In Medical Marijuana Using HPLC-PD
- FDA Memo And Guidelines For The Validation Of Chemical Methods In Food, Feed, Cosmetics, And Veterinary Products, 3rd Edition (2019)
- AOAC Official Methods Of Analysis (2002)
- APPENDIX D: Guidelines For Collaborative Study Procedures To Validate Characteristics Of A Method Of Analysis

Heavy metals

- METHOD 6020B: Inductively Coupled Plasma- Mass Spectrometry
- METHOD 6010D: Inductively Coupled Plasma - Optical Emissions Spectrometry
- METHOD 3050B, Revision 2: Acid Digestion Of Sediments, Sludges, And Soils
  METHOD 3052: Microwave Assisted Acid Digestion Of Siliceous And Organically Based Matrices
- METHOD 3031: Acid Digestion Of Oils For Metals Analysis By Atomic Absorption Or ICP Spectrometry

Residual solvents

- METHOD 8015D: Nonhalogenated Organics Using GC/FID
- METHOD 8260D: Volatile Organic Compounds By Gas Chromatography/Mass Spectrometry
- METHOD 3585: Waste Dilution For Volatile Organics
- METHOD 5021A: Volatile Organic Compounds In Various Sample Matrices Using Equilibrium Headspace Analysis

ANDREW M. CUOMO
Governor

HOWARD A. ZUCKER, M.D., J.D.
Commissioner

LISA J. PINO, M.A., J.D.
Executive Deputy Commissioner

New York State Department of Health - Wadsworth Center
Laboratory of Organic and Analytical Chemistry–
NYS ELAP Laboratory ID 10763

Division of Environmental Health Sciences
Albany, New York

Medical Marijuana Sample Preparation Protocols for Potency Analysis
NYS DOH MML-301

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1.0. Scope and Application

1.1. This method (NYS ELAP Method ID 9981) addresses the extraction of medical marijuana (MM) samples for cannabinoid analysis by High Performance Liquid Chromatography (HPLC) with Photodiode Array (PDA) Detection. It contains information specifically relevant to the extraction and preparation of MM products in **Section 8.0**. This preparation method **(NYS DOH MML-301)**, is used in conjunction with the analytical method, *Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA* **(NYS MML-300**), in support of cannabinoid analyses required per Title 10 (Health), Chapter XIII, Part 1004 of the official Compilation of Codes, Rules, and Regulations, of the State of New York. Refer to the analytical procedure (**NYS DOH MML-300**) for information on analyte list, calibration, analysis, quality control and data reporting.

1.2. This method is restricted to use by or under the supervision of analysts experienced in the preparation of MM products. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedures described in **Section 9.0**.

2.0. Summary of the Method

2.1. A portion of MM product, typically from 10 to 1200 mg, is weighed into a 50-mL centrifuge tube. The amount weighed depends upon the specific product produced by a Registered Organization (RO) and the declared concentrations of cannabinoids in the MM product. A surrogate (SUR) and typically 20.0-mL of methanol (MeOH) are added, the solution is mixed well and is either diluted further or used directly for analysis. If necessary, this extract is diluted an additional 2- to 20-fold based on the concentrations of cannabinoids in the MM sample as declared by the RO. The internal standard working diluent (IWD) is then added to the extract or dilution thereof, and the potency measurement is made using HPLC-PDA (see **NYS DOH MML-300**).

2.2. It's important to note that MM products are distinguished by brand and form (see **Section 3.0 Definitions**). Based on the current regulations, approved medical marijuana products shall be limited to the forms of administration approved by the Department, including but not limited to: metered liquid or oil preparations; solid and semisolid preparations (e.g. capsules, chewable and effervescent tablets, lozenges); metered ground plant preparations; and topical forms and transdermal patches. Medical marijuana may not be incorporated into food products by the registered organization, unless approved by the commissioner.

3.0. Definitions

3.1. Stock Standard – A concentrated solution of method analyte(s) prepared in the laboratory from referenced and certified analyte standards, where available, or a concentrated solution of method analyte(s) purchased directly from a referenced and certified source, where available.

3.2. Internal Standard (IS) – A pure compound that should not be found in any sample. The IS

is a compound added to both samples and standards at a known concentration to provide a basis for peak area ratios used in quantitation. The IS is also used to monitor instrument performance for each analysis and to correct for solvent evaporation during the analysis.

3.3. Internal Standard Stock Diluent (ISD) – A concentrated solution of IS that is prepared in solvent. This stock diluent is used to prepare the internal standard working diluent (IWD).

3.4. Internal Standard Working Diluent (IWD) – A solution of IS that is prepared from the ISD and added to all samples at the same concentration. This working diluent is used to dilute the samples and monitor the integrity of the sample injections.

3.5. Surrogate Standard (SUR) – A pure analyte, which should not be found in any sample, but is similar in nature to the compounds of interest. This compound can be added to a sample in a known amount before processing to monitor method performance for each sample.

3.6. Surrogate Stock Diluent (SSD) – A concentrated solution of SUR that is prepared in acetonitrile (MeCN). This stock diluent is used to prepare the surrogate working diluent (SWD).

3.7. Surrogate Working Diluent (SWD) – A solution of SUR that is prepared from the SSD and is added to all samples. This working diluent is used to monitor method performance.

3.8. System Blank (SBLK) – A portion of appropriate pure solvent that is analyzed to verify that the instrument is free from background contamination.

3.9. Method Blank (MB) – An aliquot of appropriate pure matrix that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents and surrogates that are used with other samples. The method blank is used to determine whether method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.10. Continuing Calibration Verification Standard (CCV) – One of the primary calibration standards used to verify the acceptability of an existing calibration.

3.11. Cross Check Reference Standard (CCR) – A solution of method standards prepared from a stock standard solution that is obtained from a source that is independent of that used to prepare the calibration standards (i.e. independent vendor, independent lot, or independent preparation). The CCR is used to verify that the original calibration source is acceptable.

3.12. Laboratory Control Sample (LCS) – A portion of appropriate clean matrix that is spiked with known quantities of target analytes and processed as a sample. The LCS measures the accuracy of the methodology. Acronyms include: Method Blank Spike (MBS) and Laboratory Fortified Blank (LFB).

3.13. Matrix Spike Sample (MS) – A portion of sample that is spiked with known quantities of target analytes and processed as if it were a sample. The sample from which the portion to be spiked was taken must be analyzed separately to determine any background analyte concentrations. The MS is corrected for background concentrations and used to determine whether the sample matrix contributes bias to the sample results. The MS is used to evaluate the accuracy of the method in the same way that the MBS is used.

3.14. Matrix Spike Duplicate Sample (MSD) – A second portion of the sample that was used to prepare the MS that is spiked and processed in an identical manner to that used for the MS. The MS and MSD are used together to measure the precision of the method.

3.15. Limit of Detection (LOD) – The statistically calculated minimum concentration of an analyte that can be measured with 99 % confidence that the value is greater than zero. Acronym: Method Detection Limit (MDL).

3.16. Limit of Quantitation (LOQ) – The minimum concentration that can be quantitatively reported for a target analyte. This limit can be no lower than the lowest calibration standard.

3.17. Preparation Batch **–** Samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch consists of one to twenty medial marijuana samples of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample.

3.18. Analytical Batch - An analytical batch consists of prepared samples which are analyzed together as a group. An analytical batch can include prepared samples originating from different matrices and can exceed twenty samples.

3.19. Brand - A defined medical marijuana product that has a homogenous and uniform cannabinoid concentration (total THC and total CBD) and product quality, produced according to an approved and stable processing protocol and shall have the same inactive ingredients as that defined for that form of the brand.

3.20. Form - A type of a medical marihuana product approved by the commissioner that shall refer to the final preparation of an approved medical marijuana brand; for example, an extract in oil for sublingual administration, an extract for vaporization or an extract in a capsule for ingestion.

3.21. Inactive ingredients - *Inactive ingredient* means any component other than an *active ingredient.*

4.0. Health and Safety Warnings

4.1. The toxicity and carcinogenicity of each chemical used in this method have not been thoroughly investigated. Therefore, each chemical compound must be treated as a potential health hazard and exposure must be limited to the lowest possible level.

4.2. Always follow guidelines listed in safety data sheets (SDS) for proper storage, handling and disposal of solvents, reagents and standards. SDSs are located within the laboratory in labeled, yellow binders. These guidelines must be made available to all personnel involved in the chemical analysis.

4.3. Lab coats, safety glasses and gloves must be worn when performing standard or sample preparations, working with instrumentation, disposing of waste and cleaning glassware.

4.4. The fume hood must be used when using or preparing standards, reagents, or samples that require proper ventilation.

4.5. The IS norgestrel is a suspected carcinogen and is a known to be hazardous during pregnancy.

5.0. Interferences

5.1. Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts observed as chromatographic peaks or elevated baselines in the chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running extracted blanks as described in **NYS DOH MML-300**.

5.2. All glassware must be washed and, if applicable, verified to be free from background contamination.

5.2.1. All new glassware and processing apparatus must be thoroughly cleaned. Before using new glassware or equipment the first time, wash with hot water and detergent, rinse with tap water and reagent water and final rinsing with methanol.

5.2.2. All routine glassware and processing apparatus must be thoroughly cleaned. After each use, rinse all glassware and processing apparatus three times with the last solvent used and dry in a clean area to prevent cross-contamination. If glassware contamination is suspected wash as per **Section 5.2.1.**

5.2.3. The use of high-purity reagents and solvents helps to minimize interference problems.

5.2.4. After cleaning, glassware is stored in a clean storage area away from standards and syringes to prevent cross-contamination.

5.3. When interferences or contamination are evident in samples, the re-preparation of the original sample is recommended after the source of contamination has been identified.

5.4. Interfering contamination known as “carry over” may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Rinsing of the sample syringe and associated equipment between samples with solvent/mobile phase can minimize this sample cross contamination. After analysis of a sample containing high concentrations of analytes one or more injections of solvent/mobile phase should be made to ensure that accurate values are obtained for the next sample.

5.5. Matrix interferences may occur due to inactive ingredients in the sample. Disclosure of inactive ingredients are sometimes held propriety by RO’s. If an inactive ingredient or other matrix interference is believed to be present, the sample may be spiked with target analytes and analyzed together with the non-spiked sample to verify the results. If these analyses verify the original results, report only the results from the original non-spiked sample. This may not always be possible if a limited amount of sample is received for analysis. If additional sample is not available for reanalysis, the original results must be qualified on the final report.

5.6. Samples and standards must be prepared in the same final solvent to allow for chromatographic comparability of samples to standards.

6.0. Equipment and Supplies

6.1. Sampling Equipment

6.1.1. Pre-cleaned 50-mL plastic bottle fitted with Teflon-lined screw cap.

6.2. Equipment

6.2.1. Analytical balance, Mettler-Toledo Model # 205DU or equivalent

6.2.2. Sonicator, Branson, Model # 2510R-DTH or equivalent.

6.2.3. Vortex, Maxi Mix 11 Model #37615 or equivalent.

6.2.4. Centrifuge, Model # 5415D or equivalent.

6.2.5. Shaker, Labline, Model# 3540 or equivalent.

6.3. Support Equipment

6.3.1. Centrifuge tubes, various sizes.

6.3.2. Stainless steel spatulas.

6.3.3. Class A volumetric flasks, various sizes.

6.3.4. Glass graduated test tubes.

6.3.5. Disposable pipettes.

6.3.6. Macro pipette controller, various sizes

6.3.7. Pipettes, pipette bulbs.

6.3.8. Aluminum foil squares and plastic weighing dishes for weighing out chemicals.

7.0. Reagents, Standards and Matrix (Consumables)

7.1. Inorganic Chemicals – Chemicals are obtained from one of the major manufactures such as Sigma-Aldrich, VWR or equivalent. All inorganic chemicals are of reagent grade quality, unless specified in **NYS DOH MML-300,** see **Section 7.0**.

7.1.1. Stable solid materials are stored in the laboratory on shelves at room temperature. Concentrated acids are also stored at room temperature in an appropriate cabinet.

7.2. Solvents – All solvents used in sample preparation must be HPLC grade (**NYS DOH MML-300,** see **Section 7.0**.). Solvents not in use are stored in solvent cabinets.

7.2.1. HPLC grade Acetonitrile (MeCN), Macron or equivalent.

7.2.2. HPLC grade Methanol (MeOH), J.T. Baker or equivalent.

7.3. Matrix Reagents- MM excipients or reagents that may be used as a “representative matrix” for matrix evaluation are listed below.

7.3.1. Medium-chain triglycerides (MCT), Warner Graham (Cat # 812N) or equivalent.

7.3.2. New matrices and excipients may also be provided by the ROs for evaluation.

7.4. Standards – Standards potency analysis are currently purchased from Cerilliant, Cayman, Restek, Sigma-Aldrich or equivalent (**NYS DOH MML-300,** see **Section 7.2**).

7.4.1. Note: Stock standard solutions or neat materials may be purchased from any vendor. When available, standards/stocks materials are purchased from vendors that can provide NIST traceability accompanied by a Certificate of Analysis.

7.5. Syringes – Syringes are obtained from one of the major manufactures such as Hamilton, SGE or equivalent. Manual syringes with fixed or removable needles are stored after cleaning. On arrival in the laboratory, new glassware is cleaned as per **Section 5.2.**

7.6. Glassware – Glassware is obtained from one of the major manufactures of laboratory glassware such as Kimble, Ace Glass, Corning or equivalent. On arrival in the laboratory, new glassware is cleaned as per **Section 5.2**.

8.0. Preparation of Reagents, Solutions, Standards, Matrices and Samples.

8.1. Standards, SUR and IS are prepared as per **NYS DOH MML-300,** see **Section 8.0.**

8.2. RO excipient materials and blank matrix (stored as per RO instructions).

8.3. Sample extract preparation procedure (including MB, MS, MSD, LCS):

8.3.1. A direct dilution method is applied for most of the MM products. This method can also be used for extraction of solid material. All samples are prepared in this manner unless problems are encountered with a specific sample matrix (i.e. form, brand). Any deviations from this sample preparation method are documented and recorded in the data packages. All recoveries are documented and recorded in the data packages. The documentation must be available for review and approval by the Department.

8.3.2. The amount of MM product to be extracted is based on the RO brand. The weight of matrix and/or medical marijuana product used is based on the concentration of cannabinoids in each product to ensure the final concentrations are within the analytical curve. The sample matrix and/or medical marijuana product extract, usually from 10 to 1200 mg, is weighed into a 50-mL centrifuge tube. Depending on form, alternate preparation steps may be required (See **MML-301-AppA**).

8.3.3. The volume of surrogate, 0.005 to 0.040 mL is spiked into the 50-mL centrifuge tube. The amount is based on cannabinoid levels in the sample reported by the RO and dilutions needed to ensure the final concentration of the SUR is within the calibration curve.

Typically, a sample that is diluted less than 5-fold will receive 5 µL of SUR standard stock solution at a concentration of 50 mg/mL as the spike into the sample. Based on the final cannabinoid concentrations, if further dilutions are necessary, the SUR is spiked into the sample at a higher concentration to ensure

that the measured concentration is within the calibration range of the SUR standard curve.

8.3.4. For extraction, add 20.0 mL of MeOH to the 50-mL centrifuge tube and, mix well for 30 minutes on a shaker to extract the sample.

8.3.4.1. The following modification for formulations requiring an aqueous extraction step may be made:

8.3.4.1.1. Add 20% water and sonicate for 15 minutes prior to surrogate and methanol addition

8.3.4.2. The following modifications may be made to increase the extraction efficiency where analysis requires using high sample volumes of the product due to low cannabinoid concentration (for example Balms containing < 1% cannabinoids):

8.3.4.2.1. Once extracted with 20 ml methanol place the samples in -20 ^{0}C freezer overnight. Afterwards remove from the freezer and sonicate the samples for 15 minutes.

8.3.4.3. The final concentration of the cannabinoids in medical marijuana extract must fall within the range of the calibration curve. In some circumstances, an additional methanol dilution of 2 to 20-fold is necessary to analyze the samples. The dilutions are determined based on the concentrations of the cannabinoids in the sample reported by the RO. A larger dilution is needed to bracket high concentration cannabinoids, while a direct injection of the extract or a less diluted sample is required for the analysis of the lower-concentration cannabinoids present in the same sample. Some samples may need to be analyzed twice to measure the primary cannabinoids.

8.3.4.4. Follow **MML-300-SOP** as per **Section 11.0** for MB, MS and MSD preparation.

8.3.4.5. Sample extracts (**section 8.3.4**) are stored in a freezer at ≤ -20°C until analysis is final. (**MML-300-SOP** see **Section 9.5)**

8.3.4.6. If necessary, transfer 1 mL of extract into a 2.0 mL centrifuge tube and centrifuge at 12,000 g for 5 min.

8.3.5. Transfer 500 µL IWD preparation @ 10 µg/mL into 2.0 mL HPLC vial (**NYS DOH MML-300 see Section 8.0**).

8.3.6. Transfer 500 µL of diluted sample supernatant prepared (Section **8.3.4)** into the HPLC vial with IWD (**Section 8.3.5**) and mix well providing a 1:1 ratio.

8.3.7. Follow **NYS DOH MML-300** as per **Section 13.0**, for sample analysis and data reporting.

9.0. Quality Control/Assurance

9.1. Demonstration of Capability (DOC)

9.1.1. All laboratory staff must perform an initial demonstration of capability in using the extraction procedures described in this SOP. The initial DOC must consist of the analysis of four or five extracted spike samples that have been fortified with all analytes of interest to a concentration of one (1) to four (4) times the LOQ. The spiking solution used must be from a source independent from those used to prepare the calibration standards.

9.1.2. The initial DOC is performed under the supervision of a trained analyst. The DOC must meet all acceptance criteria, as described in the analytical procedure **NYS DOH MML-300,** see **Section 11.0,** before the analyst may perform the procedure without supervision.

9.1.3. Annually, each analyst who will be performing the extraction method must complete a continuing DOC for each target analyte (see **NYS DOH MML-300 Table 1**). The continuing DOC may be completed by one of the following techniques if available:

9.1.3.1. Acceptable performance of a blind sample, such as an external proficiency test.

9.1.3.2. Acceptable performance of an initial DOC as described above and in **NYS DOH MML-300 (**see **Section 11.0)** at any concentration within the calibration range.

9.1.4. If major changes to the method or instrument are made, or the laboratory/analyst has not performed the method in a twelve (12) month period, each analyst must complete an initial DOC as described in **NYS DOH MML-300, Section 11.0.** Refer to **NYS DOH MML-300**, **Section 11.0**. for additional information on quality control measures, acceptance criteria and corrective actions for nonconforming data. Minor changes to the method are evaluated using the extracted spike, samples or the secondary source standard per **(NYS DOH MML-300, see Section 11.0)**.

9.2. LOD and LOQ

9.2.1. An initial LOD study for each method must be completed and documented for all target analytes in each representative matrix (see **MML-301-SOP, see Section 7.3**), on each instrument used to analyze sample extracts. If the laboratory intends to report results below the LOQ, an ongoing LOD verification is also required.

9.2.2. Based on the LOD, the laboratory shall select an LOQ that is greater than the LOD (typically 3-5x the LOD) and consistent with the needs of its client. An LOQ is required for each representative matrix, method and analyte combination. For each method, the lowest calibration standard concentration must be at or below the corresponding LOQ.

9.2.3. An initial LOQ study for each method must be completed and documented for all target analytes in each representative matrix. The initial LOD samples may be used for this purpose as long as the concentration used is at or below the LOQ. The mean recovery shall be within 70-130% of the spiked value.

9.2.4. On an ongoing basis, the laboratory shall prepare and analyze a minimum of one LOQ verification sample spiked at the same concentration as the initial LOQ verification study on each instrument during each quarter in which samples are analyzed for each representative matrix, method, and analyte combination. The recovery of the LOQ verification samples shall be within 70-130%.

9.2.5. The 2017 Method Update Rule finalized in the Environmental Protection Agency's (EPA's) Federal Register on August 28, 2017, prescribes a revised approach to Method Detection Limit (MDL)/LOD data collection and calculation per Part 136 Appendix B. The New York State (NYS) Environmental Laboratory Program (ELAP) requires that the revised procedure detailed within the EPA's document *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016* be implemented for all NYS ELAP accredited methods.

9.3. Extraction (Preparation) Batch-Specific Quality Control

9.3.1. The preparation batch size consists of a maximum of 20 medical marijuana samples (see **Section 3.17**). The following quality control samples must also be extracted, where applicable, at the prescribed frequency:

9.3.1.1. Method Blank, one (1) per preparation batch.
9.3.1.2. Matrix Spike/Matrix Spike Duplicate, one (1) each per preparation batch, if sample is provided.
9.3.1.3. Laboratory Control Sample (LCS) one (1) per preparation batch. The following may also meet the LCS requirement.

9.3.1.3.1. A laboratory control sample (LCS) may be used in place of a continuing calibration verification (CCV) (but not as a replacement for a failing CCV) for methods where the calibration goes through the same process as the LCS. Note that the more stringent acceptance criteria must be met.

9.3.1.3.2. The matrix spike may be used in place of the LCS as long as the acceptance criteria are as stringent as for the LCS.

9.3.2. Refer to the analytical procedure (**NYS DOH MML-300**) for information on the quality control measures, the applicable acceptance criteria and the corrective actions for nonconforming data.

9.4. Analytical Batch-Specific Quality Control

9.4.1. Refer to analytical procedure (**NYS DOH MML-300**) for information on quality control measures, applicable acceptance criteria and corrective actions for nonconforming data.

10.0. Data Acquisition, Reduction, Analysis and Calculations

10.1. Not applicable; refer to the appropriate analytical procedure (see **NYS DOH MML-300)**.

11.0. Sample Transport, Receipt, Preservation, Handling and Storage

11.1. Medical Marijuana Products from Registered Organizations are received, handled, verified and documented ensuring method regulatory and Accreditation Body requirements are met.

11.2. Follow instructions provided by the RO for storage prior to sample extraction.

11.3. Prior to analysis, the extracts are stored in a freezer at ≤ -20°C unless otherwise noted **(NYS DOH MML-300** see **Section 9.0.)**

11.4. Cannabinoids are light-sensitive, therefore samples must be protected from the light.

12.0. Waste Management/Pollution Prevention

12.1. Minimize solvent, chemical, reagent and standard use whenever possible to reduce the amount of hazardous waste generated.

12.2. Dispose of solvent waste in an appropriate solvent waste container, properly labeled.

12.2.1. All other solvents are separated into two categories, chlorinated and non-chlorinated and are disposed of in red, 5-Gallon solvent cans.

12.3. Dispose of non-hazardous aqueous waste in the laboratory sink followed by flushing with tap water.

12.4. Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal.

12.5. Consult federal, state and local regulations for additional information or for information on the disposal of products not described in this method.

13.0. References

13.1. Title 10 (Health), Chapter XIII, Part 1004 of the official Compilation of Codes, Rules, and Regulations, of the State of New York.

13.2. Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA (**NYS DOH MML-300**)

13.3. Definition and Procedure for the Determination of the Method Detection Limit--Revision 1.11 Environmental Protection Agency, 40 CFR (7-1-95 Edition) Part 136, Appendix B.

13.4. *Norgestrel*; MSDS No. N2260 [Online]; Sigma-Aldrich: Saint Louis MO, September 03, 2014 http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do

13.5. 21 CFR 210.3(b)(8) – Definitions. Current Good Manufacturing Practice in Manufacturing. Code of Federal Regulations. Title 21, Chapter 1, Subchapter C.

13.6. Alternate Approved Preparation Steps/ Method Validation Summary by Form (MML-301-AppA)

14.0. Appendices

Appendix A (MML-301-AppA) – Alternate Approved Preparation Steps/ Method Validation Summary by Brand/Form (see MML-301-SOP, section 8.3)

1. **Product/Form –** Metered Dose Inhaler

   a. **Method Development Narrative/Background**

      i. The metered dose inhaler (MDI) is a device typically used for inhaled respiratory medications. MDI is one of the approved forms of medical marijuana products in New York State (NYS) for the certified patients. It is a pressurized container with specific formulation in a plastic holder with a mouthpiece. When sprayed, it gives a reliable, consistent dose of medication. Our current sample preparation method is not suitable for preparation of MDI testing samples. Herein we developed a new procedure to address MDI sample preparation.

      ii. The alternate preparation avoids potential contamination during the transfer of the product (i.e. metal particulate contamination) and allows quicker sampling of product by the separate labs (organic chemistry, inorganic chemistry, and microbiology).

      iii. Following the sample preparation steps listed below, five duplicates sample were prepared from Inhaler # 2.

      iv. The Medical Marijuana Laboratory (MML) also prepared two other preparations as benchmarks (the controls) to compare against the alternative procedure proposed below.

      v. Benchmark # 1 involved preparing Inhaler # 1 by the procedure sent from the RO (cooling, puncturing, warming, pouring into vial, prep as a liquid). For this calculation, 17.6 mg is used as the dose (1.848 g EtOH mix per can ÷ 105 doses per can).

      vi. Benchmark # 2 involved preparing Inhaler # 2 in the finalized alternative procedure outlined below, except the inhaler was sprayed into a small zip-lock style bag (50 x 75 mm). The flexible bag seals around the inhaler mouthpiece and inflates to trap the gas/alcohol/distillate. The bag is then rinsed with 10 mL Methanol (MeOH) twice, then fully submerged in the solvent in the tube. The second benchmark provides a best-case preparation as the entire spray is contained within the bag.

b. Analysis of Results:

i. Table 1 – Summary of Results

Table 1. Prep	**1x (mg)**	**2x (mg)**	**3x (mg)**	**4x (mg)**	**5x (mg)**	**Average**	**%RSD**	**% of Benchmark 1 (RO Prep)**
BM 1 (RO)	4.53	--	--	--	--	**4.53**	**--**	**--**
BM 2 (Bag)	4.66	--	--	--	--	**4.66**	**--**	**102.9%**
Inhaler 2 (Tube)	4.08	4.82	4.56	4.58	4.73	**4.55**	**6.3%**	**100.5%**

ii. Table 1 lists the measured results of the potency (THC, mg/dose). The finalized procedure is within 5% of both the RO preparation and the best-case preparation. There should not be a noticeable difference in preparation between these methods. The laboratory then investigated the reproducibility between different devices and analysts, and since we already have data on the extraction method, the reproducibility was determined only from the mass dispensed. Using two analysts, they each sprayed a single actuation from inhalers 2, 3, 4, and 5, repeating five times per inhaler (20 weights per analyst, 40 overall). Analyst 1 sprayed an average mass of 46.8 mg ± 1.46 (3.1 %RSD). Analyst 2 sprayed an average mass of 47.1 mg ± 1.19 (2.5 %RSD). The relative percent difference between the two averages was 0.65%. There is not a noticeable difference between inhalers (%RSD <5%) or between analysts (%RPD <5%).

c. Procedure:

i. For Metered Dose Inhalers (MDI) where part of the formulation includes a gas (EX: Freon) a mass of material removed from the inhaler is used, not a measured mass of collected material.
ii. Shake the inhaler and prime the unit at least twice by spraying into a waste container. If the inhaler has already been used for testing, priming is not necessary.
iii. Place the inhaler on a balance and tare it. Shake and spray a single actuation into a tube by placing the mouthpiece within the opening of the tube, and keeping the inhaler upright, in an "L" shape. Cap quickly after spraying.
iv. Weigh the inhaler on the tared balance (the value will be negative). The absolute value of this mass is the value used for calculating potency, not the mass of sample in the tube

2. Product/Form - Chewables

a. Method Development Narrative/Background

i. Matrices that contain methanol insoluble materials prevent a challenge for good recovery of cannabinoids during sample preparation in the MML-300/301 methods. Products containing excipients which are highly water soluble, but mostly methanol insoluble are common. This alternate procedure was developed to address the sample preparation of such methanol insoluble preparations.

ii. A roughly 30% cannabinoids by weight 1:1 THC:CBD vape oil preparation of accurately determined concentration by the analytical method (MML-300) was used as the source of cannabinoids for spiking. The roughly 5-g chewable dose forms were cut in half length-wise with a razor blade to afford ~2.5 g samples of matrix. Three sets of five samples each were prepared for this study. The first set were samples containing matrix (~2.5 g). The low-spike set of samples contained matrix (~2.5 g) and were spiked with an accurately weighed amount of vape oil around (~7 mg). The high-spike set of samples containing matrix (~2.5 g) were spiked with an accurately weighed amount of vape oil (~35 mg). The low spike represents ~2 mg of both THC and CBD, and the highest spike is just above the 10 mg per dose limit of total THC allowed under current regulation.

b. Analysis of Results:

i. Initial data on the extracted samples was collected and processed as described in method MML-300. The surrogate recovery was 89% and the precision was <2.0% CV at all spike levels. The low-spike level showed nearly quantitative recovery for both total THC and total CBD. The standard deviation was moderate with a %CV of around 7%. The recovery fell slightly for the high-spike samples to 95.8 and 87.9% for total THC and total CBD respectively, while the precision improved to under 2% CV for both.

ii. The same samples were retested after 24 hours on the autosampler at about 4 °C. The recovery remained constant within experimental variation, while the precision decreased slightly to 8.7% and 7.6% for total THC and total CBD respectively. The surrogate recovery was constant at around 89% in all cases and a precision of <2.8% CV at all spiking levels. This result demonstrates the stability of the sample in the cooled autosampler for 24 hours.

iii. A fresh dilution of the initial extraction after being stored at 4 °C for 5 days was prepared and analyzed according to MML-300. All data for surrogate, total THC, and total CBD recoveries and precision %CV from these samples were unchanged from the initial preparation. This result demonstrates a 5-day storage stability at 4 °C after initial sample preparation.

iv. Two preparations of fully-formulated chewable products were tested by two separate analysts (one analyst per product) and found to be within ±10% of the manufacturer's laboratory value. On Formulation #1 (1:1 product), the

differences between the laboratories for total THC and total CBD were 2.7% and -6.6%, respectively. For Formulation #2, a high-THC low-CBD product, the differences between the laboratories for total THC and total CBD were -2.3% and -2.0% respectively. This agreement is within the ±10% acceptable range and is acceptable to comply with NYS Medical Marijuana Regulation 1004.11(c)(3).

c. Procedure:

i. A weighed-portion of homogenous chewable is cut lengthwise with a razor blade and added to a 50-mL centrifuge tube.
ii. Surrogate, SUR, 0.040 mL is spiked into a clean, 50-mL centrifuge tube. The amount of surrogate is based on cannabinoid levels in the sample reported by the RO and dilutions needed to ensure the final concentration of the SUR is within the calibration curve.
iii. Add 1.0 mL of Water and 19.0 mL of MeOH to the 50-mL centrifuge tube. Cap the tube tightly and sonicate at 55 °C for 15 minutes. Remove the tube and vortex for 10 seconds. Repeat. Immediately transfer about 2.0 mL of the well-mixed dispersion to a 2.0-mL centrifuge tube and centrifuge at 12,000 rpm for 5 minutes.
iv. Transfer the supernatant to a new 2.0-mL centrifuge tube and place in –20 °C freezer for >8 hours. Remove from freezer and immediately centrifuge at 12,000 rpm for 5 min. Remove supernatant and dilute with MeOH to the desired ratio based on the expected cannabinoid content as required for MML-300.

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Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA
NYS DOH MML-300

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1.0. Scope and Application

1.1. This method (NYS ELAP Method ID 9980) is to be used for the analysis of cannabinoid profiles in medical marijuana (MM) products. The method is for the determination of concentrations of the cannabinoids listed below (**Table 1**) as required by the New York State (NYS) medical marijuana regulations delineated in 10NYCCR § 1004.11(c)(2).

Table 1. Analyte List

Analyte	CAS Number	LOQ[1] MCT Matrix (µg/mL)
Cannabichromene (CBC)	20675-51-8	0.60
Tetrahydrocannabivarin (THCV)	31262-37-0	0.60
*Cannabidiolic Acid (CBDA)	1244-58-2	0.60
*Tetrahydrocannabinolic Acid (THCA)	23978-85-0	1.00
Cannabigerol (CBG)	25654-31-3	0.60
Cannabinol (CBN)	521-35-7	0.60
*Cannabidiol (CBD)	13956-29-1	0.60
*Delta-9-Tetrahydrocannabinol (Delta-9-THC)	1972-08-3	0.60
*Delta-8-Tetrahydrocannabinol (Delta-8-THC)	5957-75-5	0.90
Cannabidivarin (CBDV)	24274-48-4	0.60
4-Pentylphenyl 4-Methylbenzoate (Surrogate)	50649-59-7	0.60
Norgestrel (Internal Standard)	6533-00-2	N/A

[1] The Limit of Quantitation (LOQ) is the lowest concentration that can be accurately quantified for a target analyte (**Section 3.15**). LOQs were determined with medium-chain triglycerides (MCT) as the matrix. LOQs referenced within **Table 1** are subject to change based on LOD/LOQ determinations detailed within **Section 11.2**.
*Major analytes of interest see **Section 11.1.1.1.2**

1.2. This method is restricted to use by or under the supervision of analysts experienced in the use of high-performance liquid chromatography with photodiode array detection (HPLC-PDA) and the interpretation of ultra-violet (UV) spectra. Each analyst must demonstrate the ability to generate acceptable results with this method using the procedures described in **Section 11.1**

1.3. This procedure covers only the analysis of phytocannabinoids by using HPLC-PDA. It does not contain procedures relevant to sample extraction or the purification of sample extracts. Details of sample preparation are contained in **MML-301-SOP**.

2.0. Summary of the Method

2.1. Samples from each lot of MM product are diluted/dissolved with organic solvents (See **MML-301-SOP** for sample preparation details). The diluted samples fortified with internal standard (IS) are injected onto an HPLC. The targeted analytes are separated and subsequently detected online by monitoring UV absorbance using a PDA detector. The separation of nine cannabinoids is achieved on a C18 reversed-phase column 150 mm in length. Based on the summary data provided in **Table 1**, the limit of quantification (LOQ) for most of the cannabinoids is approximately 0.60 µg/mL. This method can be used to quantify the cannabinoid components that are present as low as 0.04% (percent by weight; the actual values for various MM products are dependent on how much material is used for testing) in the MM products.

2.2. Based on the current regulations, approved medical marijuana products shall be limited to the forms of administration approved by the Department, including but not limited to: metered liquid or oil preparations; solid and semisolid preparations (e.g. capsules, chewable and effervescent tablets, lozenges); metered ground plant preparations; and topical forms and transdermal patches. Medical marijuana may not be incorporated into food products by the registered organization, unless approved by the commissioner.

3.0. Definitions

3.1. Stock Standard – A concentrated solution of method analyte(s) prepared in the laboratory from referenced and certified analyte standards, where available, or a concentrated solution of method analyte(s) purchased directly from a referenced and certified source, where available.

3.2. Internal Standard (IS) – A pure compound that should not be found in any sample. The IS a compound added to samples, standards and quality-control samples at a known concentration to provide a basis for peak area ratios used in quantitation. The IS also used to monitor instrument performance for each analysis and to correct for solvent evaporation during the analysis.

3.3. Internal Standard Working Diluent (IWD) – A solution of IS that is prepared from the IS that is added to all samples at the same concentration. This working diluent is used to dilute the samples and to monitor the integrity of the sample injections.

3.4. Surrogate Standard (SUR) – A pure compound that should not be found in any sample but is similar in nature to the compounds of interest. This compound can be added to a sample in a known amount before processing to monitor method performance for each sample. It is quantified in a manner analogous to that used for the analytes. The SUR is useful in ensuring that there were no problems in sample preparation.

3.5. Surrogate Stock Diluent (SSD) – A concentrated solution of SUR that is prepared in MeCN. This stock diluent is used to prepare the surrogate working diluent (SWD).

3.6. Surrogate Working Diluent (SWD) – A solution of SUR that is prepared from the SSD that is added to all samples. This working diluent is used to monitor method performance.

3.7. System Blank (SBLK) – A portion of appropriate pure solvent that is analyzed to verify that the instrument is free from background contamination.

3.8. Method Blank (MB) – An aliquot of appropriate pure matrix that is treated exactly as if it were a sample, including exposure to all glassware, equipment, solvents, reagents and SUR that are used with other samples. The method blank (MB) is used to determine whether method analytes or other interferences are present in the laboratory environment, reagents or apparatus.

3.9. Calibration Standard (CalS) – A solution of method analytes prepared from stock or working standard solutions used to calibrate the instrument response with respect to analyte concentration.

3.10. Continuing Calibration Verification Standard (CCV) – One of the primary calibration standards used to verify the acceptability of an existing calibration.

3.11. Cross Check Reference Standard (CCR) – A solution of method standards prepared from a stock standard solution that is obtained from a source that is independent of that used to prepare the calibration standards (i.e. independent vendor, independent lot, or independent preparation). The CCR is used to verify that the original calibration source is acceptable.

3.12. Laboratory Control Sample (LCS) – A portion of appropriate pure matrix that is spiked with known quantities of target analytes and processed as if it were a sample. The LCS is used to evaluate the accuracy of the methodology. Acronyms include: Method Blank Spike and Laboratory Fortified Blank.

3.13. Matrix Spike Sample (MS) – An aliquot of sample prepared, taken through all sample preparation and analytical steps of the procedure unless otherwise noted in a referenced method, by adding a known amount of target analyte to a specified amount of sample for which an independent test result of target analyte concentration is available. Matrix spikes are used, for example, to determine the effect of the matrix on a method's recovery efficiency. When sample is not suitable, a "representative" matrix may be used instead. Synonym: Laboratory Fortified Sample Matrix.

3.14. Matrix Spike Duplicate Sample (MSD) – A second portion of an actual sample that was used to prepare the MS and is spiked and processed in an analogous manner to the MS. The MS and MSD are used together to determine the precision of the methodology.

3.15. Lower Limit of Quantitation (**LOQ)** – The minimum concentration that can be quantitatively reported for a target analyte. For routine analyses, the lowest calibration

standard must be at or below the **LOQ** for each analyte. **LOQ** is typically 3-5 times the **LOD**. Synonym: Method Reporting Limit (MRL).

3.16. Limit of Detection (LOD) – The statistically calculated minimum concentration of an analyte that can be measured with 99% confidence that the value is greater than zero. Acronym: Method Detection Limit.

3.17. Demonstration of Capability (DOC) – a procedure to establish the ability of the analyst to generate acceptable accuracy and precision using the method.

3.18. Preparation Batch – Samples that are prepared and/or analyzed together with the same process and personnel, using the same lot(s) of reagents. A preparation batch consists of one to twenty samples (not including method blanks, LCS, matrix spikes and matrix duplicates) of the same matrix with a maximum processing time of twenty-four (24) hours between the first and last sample.

3.19. Analytical batch – An analytical batch consists of prepared samples which are analyzed together as a group. An analytical batch can include prepared samples originating from different matrices and can exceed twenty samples.

4.0. Health and Safety Warnings

4.1. The toxicity and carcinogenicity of each chemical used in this method have not been thoroughly investigated. Therefore, each chemical compound must be treated as a potential health hazard, and exposure must be limited to the lowest possible level.

4.2. Always follow guidelines listed in safety data sheets (SDS) for proper storage, handling and disposal of solvents, reagents and standards. SDSs are located within the laboratory in labeled, yellow binders. These guidelines must be made available to all personnel involved in the chemical analysis.

4.3. Lab coats, safety glasses and gloves must be worn when performing standard or sample preparations, working with instrumentation, disposing of waste and cleaning glassware.

4.4. The fume hood must be used when using or preparing standards, reagents, or samples that require proper ventilation.

4.5. The IS, norgestrel, is a suspected carcinogen and is a known to be hazardous during pregnancy**.**

5.0. Interferences

5.1. Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts observed as

chromatographic peaks or elevated baselines in the chromatograms. All reagents and apparatus must be routinely demonstrated to be free from interferences under the conditions of the analysis by running extracted blanks as described in **Section 11.4**.

5.2. All glassware must be washed and, if applicable, verified to be free from background contamination.

5.2.1. All new glassware and processing apparatus must be thoroughly cleaned. Before using new glassware or equipment the first time, wash with hot water and detergent, rinse with tap water and reagent water, and perform a final rinse with methanol.

5.2.2. All routine glassware and processing apparatus must be thoroughly cleaned. After each use, rinse all glassware and processing apparatus three times with the last solvent used and dry in a clean area to prevent cross-contamination. If glassware contamination is suspected, wash per **Section 5.2.1.**

5.2.3. The use of high-purity reagents and solvents helps to minimize interference problems.

5.2.4. After cleaning, glassware is stored in a clean storage area away from standards and syringes to avoid cross-contamination.

5.3. When interferences or contamination are evident in samples, the re-preparation of the original sample is recommended after the source of contamination has been identified and eliminated, if possible.

5.4. Interfering contamination known as "carryover" may occur when a sample containing low concentrations of analytes is analyzed immediately following a sample containing relatively high concentrations of analytes. Rinsing of the sample syringe and associated equipment between samples with system blank can minimize this sample cross contamination. After analysis of a sample containing high concentrations of analytes one or more injections of solvent/mobile phase should be made to ensure that accurate values are obtained for the next sample.

5.5. Matrix interferences may occur because of excipients present in the sample. If a matrix interference is believed to be present, the sample may be spiked with target analytes and analyzed together with the nonspiked sample to verify the results. If these analyses verify the original results, report only the results from the original nonspiked sample. This may not always be possible if a limited amount of sample is received for analysis. If additional sample is not available for reanalysis, the original results must be qualified on the final report.

5.6. Samples and standards must be prepared in the same final solvent to allow for chromatographic comparability of samples to standards.

5.7. See **Appendix A (MML-300-AppA)** for examples of blanks, compound retention times and elution order.

6.0. Instrumentation, Equipment and Supplies

(Vendors and catalog numbers are included for illustration only. These are examples of the products currently used in the laboratory. This is not a fully inclusive list, and inclusion should not imply product endorsement. Instrumentation, equipment and supply substitutions may be made provided that the substitutions meet the method criteria. Refer to MML-301-SOP for extraction related equipment and supplies.)

6.1. Standard and Sample Preparation Equipment

6.1.1. Syringes, various sizes.

6.1.2. Eppendorf pipets, various sizes.

6.1.3. Disposable Eppendorf tips, various sizes.

6.1.4. Positive pipet, Handy Step S.

6.1.5. Positive pipet tips of various sizes.

6.1.6. Centrifuge tubes, various sizes.

6.1.7. Class "A" volumetric flasks with stoppers, various sizes.

6.1.8. Disposable glass pipettes and bulbs.

6.1.9. 2-mL autosampler vials with Teflon-lined screw caps or vials with crimp-top caps.

6.2. Instrumentation

6.2.1. Analytical balance, Mettler-Toledo Model # 205DU

6.2.2. Sonicator, Branson, Model # 2510R-DTH.

6.2.3. Vortex, Maxi Mix 11 Model #37615.

6.2.4. Centrifuge, Model # 5415D.

6.2.5. Shaker, Labline, Model# 3540.

6.2.6. A complete HPLC system, equipped with a column oven which is suitable for use with a variety of columns, as well as all the required accessories including:

syringes, analytical columns, gases, detectors and a data system for instrument control and data analysis/processing.

6.2.6.1. Components of the Shimadzu HPLC system used:

6.2.6.1.1. Micro vacuum degasser, model # DGU-20A3.

6.2.6.1.2. Solvent selector model# FCV-11A2**.**

6.2.6.1.3. Pumps, model # LC-20ADxR.

6.2.6.1.4. Column oven, model # CTO-20A.

6.2.6.1.5. Autosampler, model # SIL-20ACxR.

6.2.6.1.6. System controller, model # CBM-20A.

6.2.6.1.7. Photodiode array detector, model# SPD-M20A.

6.2.6.1.8. Operating software, Shimadzu LabSolutions.

7.0. Reagents and Standards

7.1. Solvents (HPLC Grade) and reagents – All solvents and reagents must have records that trace their origins and preparations, including Certificates of Analysis, laboratory receipts and preparation records.

7.1.1. Methanol (MeOH) – HPLC grade.

7.1.2. Acetonitrile (MeCN) – HPLC grade.

7.1.3. Water – HPLC grade.

7.1.4. Acetone – HPLC grade.

7.1.5. Ammonium formate – 98+ % purity, Fluka catalog # 3272-02.

7.1.6. Formic acid – MSD grade, Sigma-Aldrich catalog # 39,938-8.

7.1.7. Miglyol – Miglyol 812, Warner Graham, catalog # 140325.

7.2. Stock standards

7.2.1. When available, stock standards are purchased from vendors who can provide NIST- traceable standards accompanied by Certificates of Analysis.

7.2.2. The commercial standards/materials listed in **Sections 7.2.4** (**Table 2**), **7.2.5** (**Table 3**), **7.2.6** (**Table 4**), **7.2.7** (**Table 5**) and **7.2.8** (**Table 6**) are examples of those currently used in the laboratory, alternate vendors may be used. This is not a fully inclusive list, and substitutions may be made if the criteria described above are met.

7.2.3. At a minimum, commercial standards/materials are stored per the manufacturer's recommended storage conditions and expiration dates are as prescribed by the vendor on their Certificate of Analysis.

7.2.4. Cerilliant analytical reference standards

Table 2.

Standard	Catalog #	Concentration	Solvent
CBN	C-046	1.0 mg/mL	MeOH
CBD	C-045	1.0 mg/mL	MeOH
Delta-9-THC	T-005	1.0 mg/mL	MeOH
Delta-8-THC	T-032	1.0 mg/mL	MeOH
CBG	C-141	1.0 mg/mL	MeOH
CBDV	C-140	1.0 mg/mL	MeOH
CBC	C-143	1.0 mg/mL	MeOH
CBDA	C-144	1.0 mg/mL	MeCN
THCV	T-094	1.0 mg/mL	MeOH
THCA	T-093	1.0 mg/mL	MeCN

7.2.5. Cayman Chemical analytical reference standard

Table 3.

Standard	Catalog #	Concentration	Solvent
CBN	ISO60183	1.0 mg/mL	MeOH
CBD	ISO60156	1.0 mg/mL	MeOH
Delta-9-THC	ISO60157	1.0 mg/mL	MeOH
Delta-8-THC	ISO60158	1.0 mg/mL	MeOH
CBG	20164	1.0 mg/mL	MeOH
CBDV	20165	1.0 mg/mL	MeOH
CBC	ISO60163	1.0 mg/mL	MeOH
CBDA	18090	1.0 mg/mL	MeCN
THCV	18091	1.0 mg/mL	MeOH
THCA	ISO60175	1.0 mg/mL	MeCN

7.2.6. Restek analytical reference standards

Table 4.

Standard Name	Catalog #	Concentration	Solvent
CBN	34014	1000 µg/mL	MeOH
CBD	34014	1000 µg/mL	MeOH
Delta-9-THC	34014	1000 µg/mL	MeOH

7.2.7. Sigma-Aldrich analytical reference standards

Table 5.

Standard	Catalog #	Concentration	Solvent
4-pentylphenyl 4-methybenzoate (SUR)	665754	n/a	Solid

7.2.8. Fluka analytical reference standards

Table 6.

Standard	Catalog #	Concentration	Solvent
Norgestrel (IS)	10006319	n/a	Solid

8.0. Preparation of Reagents, Solutions and Standards

8.1. General preparation information

8.1.1. All reagents, solutions and standards must be traceable to stocks and, when available, have NIST-traceable documentation. The preparation method, date of preparation, expiration date and analyst must also be traceable in laboratory documentation.

8.1.2. Standard preparation steps are offered for guidance only. Alternate preparations, concentrations and stock mixtures may be utilized provided that they meet the requirements detailed herein.

8.1.3. A syringe or positive pipet is used to deliver any volume of sample or standard that will be quantified in the analysis. Eppendorf pipets are used for transferring volumes only when quantification is not necessary. Replace pipet tips after each solution change.

8.2. IWD Preparations (from neat)

8.2.1. IWD preparation from neat standard @ 10 µg/mL. The IWD is prepared annually and stored at -20 °C.

8.2.1.1. The IWD is prepared directly from the neat material.

8.2.1.1.1. Weigh 10.0 mg of norgestrel (IS) into a 1-L volumetric flask.

8.2.1.1.2. Dilute to volume with MeOH.

8.2.1.1.3. Sonicate for 1-2 min or until all solids are in solution.

8.2.1.1.4. Invert several times to mix well and transfer to Wheaton bottles for storage.

8.3. SSD @ 50 mg/mL. The SSD is prepared annually and stored at -20 °C.

8.3.1. The SSD is prepared by weighing 500 mg of 4-pentylphenyl 4-methylbenzoate into a weighing vessel and transferring it into a 10-mL volumetric flask containing MeCN. The volumetric is then diluted to volume with MeCN.

8.3.2. The SSD is mixed well and labeled appropriately.

8.4. SWD @ 100 µg/mL. The SWD is prepared monthly and stored at -20 °C.

8.4.1. Transfer 200 µL of SSD prepared in **Section 8.3** into a 100-mL volumetric flask.

8.4.2. Fill to volume with MeOH, mix well and label.

8.5. Primary cannabinoid standard stock solution @ 90 µg/mL. The Primary cannabinoid standard is prepared annually and stored at -80 °C.

8.5.1. Aliquots (0.9 mL) of each standard solution (1000 µg/mL) purchased from vendors are added to a 10-mL volumetric flask.

8.5.2. Fill to volume with MeOH and invert 3 times to mix.

Table 7. (*The primary cannabinoid standard mixture is prepared in a 10-mL volumetric flask as a combined preparation)*

Standard number	Volume Added	Standard name	Catalog #	Diluted Concentration
1	0.9 mL	CBN	C-046	90 µg/mL
2	0.9 mL	CBD	C-045	90 µg/mL
3	0.9 mL	Delta-9-THC	T-005	90 µg/mL

4	0.9 mL	Delta-8-THC	T-032	90 µg/mL
5	0.9 mL	CBC	C-143	90 µg/mL
6	0.9 mL	THCV	T-094	90 µg/mL
7	0.9 mL	CBDA	C-144	90 µg/mL
8	0.9 mL	THCA	T-093	90 µg/mL
9	0.9 mL	CBG	C-141	90 µg/mL
10	0.9 mL	CBDV	C-140	90 µg/mL

8.6. Primary cannabinoid working solution and surrogate @ 45.0 µg/mL. The primary cannabinoid working solution is prepared annually and stored at -80 °C.

8.6.1. Take 1.00 mL of the primary cannabinoid stock solution at 90 µg/mL prepared in **Section 8.5,** place into a 2-mL volumetric flask and add 0.900 mL of SWD prepared in **Section 8.4.**

8.6.2. Fill to volume with MeOH and invert 3 times to mix.

8.7. A cross check reference standard (CCR) stock standard prepared as a secondary and separate cannabinoid stock solution using Cayman standards is prepared at 9.0 µg/mL. The CCR is prepared annually and stored at -80 °C.

Table 8. CCR (*This CCR is prepared in a 25-mL volumetric as a combined preparation)*

Standard number	Volume Added	Standard name	Catalog #	Diluted Concentration
1	0.225 mL	CBN	C-046	9.0 µg/mL
2	0.225 mL	CBD	C-045	9.0 µg/mL
3	0.225 mL	Delta-9-THC	T-005	9.0 µg/mL
4	0.225 mL	Delta-8-THC	T-032	9.0 µg/mL
5	0.225 mL	CBC	C-143	9.0 µg/mL
6	0.225 mL	THCV	T-094	9.0 µg/mL
7	0.225 mL	CBDA	C-144	9.0 µg/mL
8	0.225 mL	THCA	T-093	9.0 µg/mL
9	0.225 mL	CBG	C-141	9.0 µg/mL
10	0.225 mL	CBDV	C-140	9.0 µg/mL

8.7.1. Portions (0.225 mL) of each standard solution as purchased from vendors (1000 µg/mL) are added to a 25-mL volumetric flask.

8.7.2. Fill to volume with MeOH and invert 3 times to mix.

8.8. As needed, an additional CCR standard (Restek) is prepared @ 10 µg/mL to verify the calibration curve. The CCR (Restek) is prepared annually and stored at -80 °C.

The Restek stock standard contains only 3 Cannabinoids at 1000 µg/mL. Since this stock contains only 3 cannabinoids, it is used, when necessary, in addition to the CCR (Cayman) prepared in **Section 8.7**.

Table 9.

Standard Number	Volume used	Standard Name	Catalog #	Diluted Concentration
1	10 µL	CBN	34014	10 µg/mL
2	10 µL	CBD	34014	10 µg/mL
3	10 µL	Delta-9-THC	34014	10 µg/mL

8.8.1. 0.01 mL of standard solution mix as purchased from vendor at 1000 µg/mL is added to a 10-mL volumetric flask.

8.8.2. Fill to volume with MeOH and invert 3 times to mix.

8.9. Mobile Phases. The mobile phases are maintained at room temperature and must be prepared monthly.

8.9.1. HPLC mobile phase A: 25 mM ammonium formate, 0.1% formic acid in HPLC water.

8.9.1.1. 1.575 g of ammonium formate is added to a scintillation vial and dissolved with a small volume of HPLC grade water.

8.9.1.2. Ammonium formate solution prepared in **Section 8.9.1.1** is transferred to a 1-L volumetric flask. The scintillation vial is rinsed well with HPLC grade water and 1.0 mL of formic acid is added to the 1-L volumetric flask. Dilute to volume with HPLC grade water and mix well.

8.9.2. HPLC mobile phase B: MeCN containing 0.1% formic acid.

8.9.2.1. Add 1.0 mL of formic acid into a 1-L volumetric flask. Add MeCN to a total volume of 1-L and mix well.

8.9.3. Matrices – stored as per Manufacturer's recommendations.

Table 10.

Matrices	**Manufacturer**	**Catalog #**
Miglyol – Oil based matrix	Warner Graham Co	140325

8.9.3.1. MCT is used as a representative matrix in the preparation of DOC, LOD, LOQ, MS and MSD.

9.0. Shipping Conditions, Receiving, Preparation, Analysis and Storage

9.1. Sample shipping conditions

9.1.1. The MM products from the Registered Organizations (RO) are shipped per manufacturer's specifications and must adhere to all regulatory requirements.

9.2. Sample receiving

9.2.1. Medical marijuana products from the RO are received, verified and documented ensuring that method, regulatory and Accreditation Body requirements are met.

9.2.2. All MM products must be stored under the conditions based on the manufacturer's recommendation. The storage conditions are documented.

9.2.3. All MM products must be stored under the conditions based on the manufacturer's recommendation. The storage conditions are documented.

9.3. Sample preparation

9.3.1. Follow MM sample preparation as per **MML-301-SOP** and document preparation of all samples.

9.4. Sample analysis:

9.4.1. Samples for analysis are placed into the autosampler, which is maintained at 4-10 °C. Samples are analyzed by HPLC-PDA using a Poroshell C18 column. Ultraviolet (UV) absorption spectra are recorded over the wavelength range of 190 to 800 nm. The absorbance at 227 nm is displayed to provide a chromatogram of the peaks representing the cannabinoid components, which are then integrated for analyte quantitation. Chromatography is achieved using the mobile phases described in **Section 8.9** and the instrumental parameters as outlined in **Tables 11** and **12**.

9.4.2. HPLC analytical parameters

9.4.2.1. Injector

9.4.2.1.1. The Injection volume is 10-µL.

9.4.2.2. Mobile phases

9.4.2.2.1. Mobile phase A: 0.1% formic acid in 25 mM ammonium formate (aqueous).

9.4.2.2.2. Mobile phase B: 0.1% formic acid in MeCN.

9.4.2.3. Column

9.4.2.3.1. Column: Agilent Poroshell 120, EC-C18, 3.0 x 150 mm, 2.7 µm particle size, Cat # 693975-302 or equivalent.

9.4.2.3.2. Column oven temperature: 30 °C.

9.4.2.4. HPLC conditions

9.4.2.4.1. HPLC: Flow rate of 0.625 mL/min with a gradient of mobile phase A/mobile phase B composition as shown in the **Table 11** for the Agilent Poroshell 120 column (the gradient may be modified depending on the column used).

9.4.2.4.2. These parameters serve as a guideline and may be adjusted to optimize separation if the quality performance criteria are met in **Section 11.0.**

Table 11. Mobile phase gradient

Time (min)	%Mobile A	%Mobile B
0.0	27	73
18.00	27	73
19.00	0	100
21.00	0	100
22.10	27	73
25.00	27	73

9.4.2.4.3. Data collection time: 18.0 min.

9.4.2.4.4. Total run time: 25.00 min.

9.4.2.5. PDA Detector

9.4.2.5.1. Wavelength scan range: 190 - 800 nm.

9.4.2.5.2. Wavelength for display and peak integration: 227 nm (If necessary, an alternate wavelength may be used).

9.5. Extract storage

9.5.1. Sample extracts (20 mL) are stored in a freezer at ≤ -20 °C until analysis, which must be completed within 7 days of extraction. After testing is completed, the remaining extracts are stored at ≤ -20 °C for one month for reanalysis if it is necessary.

9.6. Mobile phase storage

9.6.1. When they are maintained at room temperature, it is common practice to dispose of any aqueous mobile phases after one month. This is to prevent any microbial growth and changes in the mobile phases.

10.0. Calibration

10.1. Initial calibration

Examples of currently used instrumental integration parameters are listed below (see **Table 12)**. Results are calculated using peak area. These settings serve as guidelines and may be adjusted for optimization of integration. If interferences preclude use of IS (norgestrel), an external standard calibration may be used to calculate the results (see **Section 13.3 and Section 14.11**) provided that other quality performance criteria are achieved (see **Section 11.0**).

Table 12. Integration parameters for a calibration curve using absorbance at 227 nm wavelength

Description	Units	Value
Channel	nm	227
Width	s	1
Slope	μV/min	15500
Drift	μV/min	0
Time to double peak	min	1000
Minimum Area/Height	counts	1000

10.1.1. The primary standard stock solution prepared in **Section 8.5** is used to prepare calibration standards for the cannabinoids at concentrations appropriate for the range of the instrument and the sample content. A minimum of 5 calibration concentrations must be analyzed for each cannabinoid.

10.1.2. The lowest level calibration standard must be at or below the LOQ values listed in **Section 1.0, Table 1** for each analyte, or the LOQ values must be adjusted accordingly.

10.2. Primary cannabinoid stock standard curve preparation. The calibration curve standards are prepared monthly and stored at -20 °C for up to 2 months.
Concentration range for all compounds including the surrogate is 45.0 µg/mL to 0.46 µg/mL. It is highly recommended that positive pipets or glass syringes are used for calibration curve preparation (See **Table 13** for dilution schedules).

10.2.1. ***CalS-6* 45.0 µg/mL**

10.2.1.1. Fill one 1.5-mL centrifuge tube with 1.00 mL of primary standard stock solution prepared in **Section 8.5** and surrogate prepared in **Section 8.4**. This solution is labeled as **CalS 6**.

10.2.2. ***CalS-5* 18.0 µg/mL**

10.2.2.1. Transfer 400 µL **CalS 6** taken from the initial centrifuge tube in (see **Section 10.2.1.1)** into **CalS 5** vial.
10.2.2.2. Add 600 µL MeOH.
10.2.2.3. Mix well.

10.2.3. ***CalS-4* 7.20 µg/mL**

10.2.3.1. Transfer 400 µL **CalS 5** taken from the HPLC vial (see **Section 10.2.2.3**) into **CalS 4** vial.
10.2.3.2. Add 600 µL MeOH
10.2.3.3. Mix well.

10.2.4. ***CalS-3* 2.88 µg/mL**

10.2.4.1. Transfer 400 µL **CalS 4** taken from the HPLC vial (see **Section 10.2.3.3**) into **CalS 3** vial.
10.2.4.2. Add 600 µL MeOH
10.2.4.3. Mix well.

10.2.5. ***CalS-2* 1.15 µg/mL**

10.2.5.1. Transfer 400 µL **CalS 3** taken from the HPLC vial (see **Section 10.2.4.3**) into **CalS 2** vial.
10.2.5.2. Add 600 µL MeOH
10.2.5.3. Mix well.

10.2.6. ***CalS-1* 0.46 µg/mL**

10.2.6.1. Transfer 400 µL **CalS 2** taken from the HPLC vial (see **Section 10.2.5.3**) into **CalS 1** vial.
10.2.6.2. Add 600 µL MeOH
10.2.6.3. Mix well.

10.2.7. Prepare calibration standard mixtures with IWD prepared in **Section 8.2**

10.2.7.1. Following **Table 13** to prepare the standards in HPLC vials: from **CalS-IWD-1** through **CalS-IWD-6.**

Table 13. Dilution schedule of CalS-1 thru CalS-5
Initial Standard dilution schedule w/out IWD (for final standard prep in MeOH)

Cal std	Std conc ug/mL	Cannabinoid std ref. Section ID	Volume of cannabinoid std	MeOH
CalS-6	45	**Section 8.5**	-	-
CalS-5	18	**10.2.1.1**	400 µL	600 µL
CalS-4	7.2	**10.2.2.3**	400 µL	600 µL
CalS-3	2.88	**10.2.3.3**	400 µL	600 µL
CalS-2	1.15	**10.2.4.3**	400 µL	600 µL
CalS-1	046	**10.2.5.3**	400 µL	600 µL

Table 14. Final std dilution schedule mixed with IWD (for analysis on instrument).

Cal std w/IWD	Cal std (**Table 13**)	Volume of cannabinoid stock std	IWD As prepared in **Section 8.2**
CalS-IWD-6	CalS-6 (**10.2.1.1**)	500 µL	500 µL
CalS-IWD-5	CalS-5 (**10.2.2.3**)	500 µL	500 µL
CalS-IWD-4	CalS-4 (**10.2.3.3**)	500 µL	500 µL
CalS-IWD-3	CalS-3 (**10.2.4.3**)	500 µL	500 µL
CalS-IWD-2	CalS-2 (**10.2.5.3**)	500 µL	500 µL
CalS-IWD-1	CalS-1 (**10.2.6.3**)	500 µL	500 µL

10.2.8. Starting with the lowest standard concentration, analyze each calibration standard and tabulate the responses (analyte peak area/IS peak area). The results are used to prepare a calibration curve for each target analyte (weighted 1/C linear regression).

10.3. Initial calibration criteria

10.3.1. The absolute IS response in each chromatographic run must not deviate by more than 10% from average of its initial calibration values.

10.3.2. The correlation coefficient (r) of the calibration curve for each analyte must be verified to be ≥ 0.995 before any analysis of samples can begin.

10.3.3. Each calibration standard, processed under the new initial calibration, must be within 90-110% of the known value for each analyte for the initial calibration to be considered valid. Exception: standards ≤LOQ must be within 70-130%. Evaluation of each standard also serves as the measure of % Relative Error with the same acceptance criteria.

10.3.4. If these criteria cannot be met, a new calibration must be established.

10.4. Initial verification of calibration

10.4.1. The initial calibration for each cannabinoid must be verified by analyzing a CCR. The CCR is prepared as described below but higher or lower levels or volumes may be prepared. It is recommended that the CCR is prepared at a concentration within the middle of the calibration curve.

10.4.2. Cayman CCR solution (**Section 8.7**) @ 4.5 µg/mL. The CCR is stored for up to 1 year at -80 °C or up to 1 month at -20 °C.

10.4.2.1. In a 20-mL centrifuge tube, add 5 µL of SSD (**Section 8.3**) and add 20 mL of MeOH.

10.4.2.2. Add 250 µL MeOH with surrogate from **Section 10.4.2.1** into an HPLC vial.

10.4.2.3. Add 250 µL CCR standard solution @ 9.0 µg/mL from **Section 8.7** into the same vial.

10.4.2.4. Add 500 µL of IWD prepared in **Section 8.2** into the same vial and vortex well.

10.4.2.5. Label as CCR @4.5 µg/mL.

10.4.2.6. The measured recovery values for the analytes of the CCR must fall within 85-115% of the known value for the cannabinoids.

10.4.3. Restek CCR solution (**Section 8.8**) @ 5.0 µg/mL. The CCR is prepared as needed and store up to 1 year at -80 °C and up to one month at -20 °C.

10.4.3.1. The Restek CCR is prepared as described below but higher or lower levels or volumes may be prepared. It is recommended that the Restek CCR is prepared at a concentration within the middle of the calibration curve. An example of Restek CCR @ 5.0 µg/mL is prepared as follows:

10.4.3.2. Into a 1-mL volumetric add 500µL of MeOH with surrogate solution from **Section 10.4.2.1** and add 500 µL of Restek CCR at 10 µg/mL as prepared in **Section 8.8** and labeled as Restek CCR Stock.

10.4.3.3. Into a 2-mL HPLC vial, add 500µL of Restek CCR as prepared in **Section 10.4.2.10** and 500 µL of IWD in **Section 8.2**. Transfer solution to a label vial and vortex.

10.4.3.4. Label as Restek CCR @ 5.0 µg/mL. An appropriate volume for instrument analysis is transferred to labeled HPLC vials with inserts, capped and stored. Since this stock contains only 3 cannabinoids, it is used, when necessary, in addition to the CCR (Cayman) prepared in **Section 8.7**

10.4.3.5. The measured recovery values for the analytes of the Restek CCR must fall within 85-115% of the known value for CBN, CBD and Delta-9-THC.

10.5. Initial and Ongoing Calibration Verification

10.5.1. The initial calibration curve for each cannabinoid must be verified by the analysis of a mid-level CCR

10.5.1.1. The mid-level CCR must be within 85-115% of the known value for each analyte within the initial calibration.

10.5.2. After verifying the initial calibration, a CCV that is ≤ 1/2 the highest calibration standard must be analyzed with each analytical batch (typically 20 samples). For external calibration, a CCV is required at the beginning and end of each analytical batch. For internal standard calibration, a CCV is only required at the beginning of the analytical batch.

10.5.2.1. Low-level CCVs that are ≤ the LOQ must be within 70-130 % of the known value for each analyte. CCVs > the LOQ must be within 90-110% of the predicted concentration.

10.5.2.2. CCVs may also be interspersed throughout the analytical batch at varying concentrations provided that the CCVs analyzed at the beginning and end (for external calibration) of each analytical batch are equal to or less than half the highest calibration level. Additional CCVs may also be run at higher levels to evaluate the upper end of the calibration curve.

10.5.2.2.1. Examples of CCV levels are as follows: 18.0µg/mL, 7.20 µg/mL, 2.88 µg/mL and 1.15 µg/mL (see **Section 10.2**, **CalS-IWD- 5** through **CalS-IWD-2**).

11.0. Quality Control and Assurance

11.1. DOC

11.1.1. Each analyst must perform a DOC using the procedures described in this SOP for each target analyte listed in **Table 1**.

11.1.1.1. Initial DOC

11.1.1.1.1. Prior to analyzing samples, the analyst must perform an initial DOC consisting of four or five solvent-spike samples that have been fortified with all analytes of interest at a concentration of one (1) to four (4) times the LOQ. If possible, the spiking solution should be from a source independent of those used to prepare the calibration standards.

11.1.1.1.2. For each individual analyte, the recovery value for all replicates must fall within the range of 85 -115 %. The precision of the measurements, calculated as relative standard deviation (RSD), must be < 5% for the major analytes such as Delta-8-THC, Delta-9-THC or CBD and < 10 % for minor analytes. Each analyst must complete a successful initial DOC prior to analyzing samples.

11.1.1.2. Continuing DOC

11.1.1.2.1. Annually, each analyst must complete a continuing DOC for each target analyte. The continuing DOC may be completed by one of the following techniques:

11.1.1.2.1.1. Acceptable performance on the analysis of a blind sample, such as an external proficiency test, when available.

11.1.1.2.1.2. Acceptable performance on an initial DOC as described above in **Section 11.1.1.1** at any concentration within the calibration range.

11.1.2. If major changes to the method or the instrumentation are made, or the laboratory/analyst has not performed the method in a twelve (12) month period,

the analyst must complete an initial DOC as described in (**Section 11.1.1.1**). Minor changes to the method are evaluated using the LCS (**Section 11.5**).

11.1.3. All initial and continuing DOCs must be documented.

11.2. LOD and LOQ

11.2.1. An initial LOD study for each method must be completed and documented for all target analytes in each representative matrix (see **MML-301-SOP, Section 7.3**), on each instrument used to analyze sample extracts. If the laboratory intends to report results below the LOQ, an ongoing LOD verification is also required.

11.2.2. Based on the LOD, the laboratory shall select an LOQ that is greater than the LOD (typically 3-5x the LOD) and consistent with the needs of its client. An LOQ is required for each representative matrix, method and analyte combination. For each method, the lowest calibration standard concentration must be at or below the corresponding LOQ.

11.2.3. An initial LOQ study for each method must be completed and documented for all target analytes in each representative matrix. The initial LOD samples may be used for this purpose as long as the concentration used is at or below the LOQ. The mean recovery shall be within 70-130% of the spiked value.

11.2.4. On an ongoing basis, the laboratory shall prepare and analyze a minimum of one LOQ verification sample spiked at the same concentration as the initial LOQ verification study on each instrument during each quarter in which samples are being analyzed for each representative matrix, method, and analyte combination. The recovery of the LOQ verification samples shall be within 70-130%.

11.2.5. The 2017 Method Update Rule finalized in the Environmental Protection Agency's (EPA's) Federal Register on August 28, 2017, prescribes a revised approach to Method Detection Limit (MDL)/LOD data collection and calculation per Part 136 Appendix B. The New York State (NYS) Environmental Laboratory Program (ELAP) requires that the revised procedure detailed within the EPA's document *Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016* be implemented for all NYS ELAP accredited methods.

11.3. SBLK

11.3.1. Prior to beginning analysis, the analyst must demonstrate that the instrument is free from background interference by analyzing an SBLK.

11.3.2. Fill an HPLC vial with MeOH and analyze as an SBLK. Background contamination found, which could interfere with the measurement of target analytes, must be < 1/3 LOQ for routine samples.

11.4. MB

11.4.1. Before processing samples, the analyst must demonstrate that all glassware and reagent interferences are under control. For each preparation batch (1 to 20 samples of the same matrix with a maximum processing time of 24-hours between the first and last sample) or each time reagents are changed, an MB must be analyzed. If, within the retention time window of any target analyte, the MB produces a peak that would prevent the determination of the analyte, identify the source of contamination and eliminate the interference before processing the samples. Background contamination observed must be <1/3 the LOQ for each target analyte.

11.4.1.1. Fill an HPLC vial with 500 µL of blank extracted matrix and 500 µL of IWD.

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11.5. LCS

11.5.1. One LCS is required with each preparation batch (1 to 20 samples of the same matrix with a maximum processing time of 24-hours between the first and last sample). The following rules may also be applied to the LCS requirement.

11.5.1.1. A laboratory control sample (LCS) may be used in place of a continuing calibration verification (CCV) (but not as a replacement for a failing CCV) for methods where the calibration goes through the same process as the LCS. Note that the more stringent acceptance criteria must be met.

11.5.1.2. The matrix spike (MS) may be used in place of the LCS as long as the acceptance criteria are as stringent as for the LCS.

11.5.2. The LCS must be spiked with all target analytes at a mid-level concentration in the curve.

11.5.3. The recovery of the LCS must be within 80-120% of the expected prepared value.

11.5.4. The LCS is stored up to 1 year at -80 °C and up to 1 month at -20 °C.

11.6. SUR

11.6.1. The SUR is spiked into all samples. The measured concentration for the SUR in each sample should be within 80-120% of the expected prepared value.

11.7. IS

11.7.1. The IS is spiked into all samples. The IS peak area in all the analyzed samples must be within 10 % of the mean values of the initial calibration curve.

11.8. MS and MSD

11.8.1. A matrix spike and matrix spike duplicate are required with each preparation batch (1 to 20 samples of the same matrix with a maximum processing time of 24-hours between the first and last sample). If sample is not available, a "representative matrix" is used to prepare the MS/MSD and is spiked at a mid-level (4.5 µg/mL) concentration with the target analytes.

11.8.2. To determine the accuracy, calculate the percent recovery of the concentration for the analyte in the **MS**. Recovery must be within 80 – 120% of the true value.

11.8.3. To determine the precision, calculate the relative percent difference (RPD) between the MS/MSD. The RPD must be <20% (**Section 13.3.3**).

11.8.4. MS and MSD are stored at -20 °C until sample analysis.

11.9. System Performance Mix Requirements

11.9.1. Method specifications

Table 16

Test	Analyte	Concentration	Requirement
Sensitivity	CBN	CalS @ LOQ	Detection of analyte at S/N >3
Chromatographic Performance	Delta-9-THC	CalS @ LOQ	0.80 <PGF[a] < 1.15
Column Performance	Delta-9-THC and Delta-8-THC	CalS @ LOQ	[b]Resolution >1.0

[a]PGF = Peak Gaussian Factor (See Equation 3)
[b]Resolution between the two peaks (See Equation 4)

Equation 3.

$$PGF = \frac{1.83 \times W(1/2)}{W(1/10)}$$

W = (1/2) is the peak width at half height, and W (1/10) is the peak width at tenth height.

Equation 4.

$$R = t/W$$

t = the difference in elution times between the two peaks
W = the average peak width at the baseline of the two peaks.

11.9.2. Instrument specifications

11.9.2.1. An instrument performance system check is carried out daily using a standard at or below the detection limit. Documentation of this is maintained to ensure instrument hardware is functional (i.e., detectors and pumps).

11.9.2.2. Planned maintenance (PM) must be performed once a year per Manufacturer's specifications. Documentation of this must be maintained in the laboratory. The purpose of PM is to establish the initial installation and performance procedures that are required for evaluating the acceptability of the instrument's performance. PMs address immediate and future service issues on instrumentation to maximize system productivity.

12.0. Extraction Procedure

12.1. See appropriate extraction procedure (see **MML-301-SOP**) for more information

13.0. Data Acquisition, Reduction, Analysis and Calculations

13.1. HPLC

13.1.1. Other HPLC columns and/or chromatographic conditions may be used if the retention time acceptance limits are within 2%. See **Appendix A (see MML-300-AppA)** for relative retention times**.**

13.1.2. Perform an initial calibration (**see Sections 10.1 – 10.3**) or verify the initial calibration on each day of analysis by analyzing a CCV as described in **Section 10.5.2.** For all analyses the standards and sample extracts must be in MeOH.

13.1.3. If the response for a target analyte exceeds the working range of the instrument, dilute the extract in diluent and reanalyze.

13.1.4. If concentrations above the calibration curve are expected, the sample extract may be diluted and analyzed to prevent detector saturation and/or negative impacts on the column and/or injector. Since the IS is added after the dilution of the extract, there is no impact on the IS.

13.1.5. When the software inadequately integrates a peak and manual integration does become necessary, laboratory specific procedures must be used a guidance for any manual integration of peaks.

13.1.6. If interference is suspected due to carryover (**see Section 5.4**) and target cannabinoids are present in an unusually concentrated extracted sample, the analyst must demonstrate that the compounds in the subsequent sample are not due to carryover. After the analysis of a sample containing high concentrations of cannabinoids, SBLK should be analyzed to evaluate cross-contamination (**see Section 14.7**). Alternatively, if the sample immediately following the high concentration sample does not contain the cannabinoids present in the high-level sample, freedom from contamination has been established. It is the responsibility of the analyst to confirm that no peaks have carried over into a subsequent analysis thereby compromising the integrity of the analytical results.

13.1.7. A SBLK should be analyzed prior to sample analyses to ensure that the total system (i.e., syringe, lines and HPLC system) is free of contaminants. It is also recommended that new and currently in-use columns be washed with 100% mobile phase B for a period of 2 h prior to startup for the analysis of new batches.

13.2. Identification of analytes

13.2.1. Identify a sample component by comparison of its retention time with the retention time of a reference chromatogram (standard). If the retention time of an unknown compound corresponds, within limits, to the retention time of a standard compound and IS, then identification is considered positive.

13.2.2. The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of an analytical sequence. Three times the standard deviation (SD) of a retention time can be used to calculate a suggested window size for a compound. However, the experience of the analyst should weigh heavily in the interpretation of the chromatograms.

13.2.3. Current retention time windows are set to allow less than 2% deviation from the expected retention times for all analytes. The expected retention time might change slightly with extended column usage. The expected retention time for each analyte must be verified as necessary using the calibration standards.

13.2.4. Identification of analytes requires expert judgment when sample components are not resolved chromatographically or if any doubt exists over the identification of a peak on a chromatogram. If necessary, the analyst may need to employ appropriate alternate techniques to help confirm peak identification, such as alternate wavelengths and columns. Alternate methods to verify the identification of unknown peaks are under development.

13.2.5. See MML-300-AppA for example chromatograms and retention times by analyte.

13.3. Calculations

13.3.1. Evaluation of Initial Calibration Standards (external calibration)

13.3.1.1. The software calculates the recoveries for the calibration standards. Low-level CCVs that are ≤ the LOQ must be within 70-130 % of the known value for each analyte. CCVs > the LOQ must be within 90-110% of the predicted concentration.

13.3.1.2. Calculate the retention time of each standard compound in the calibration curve. The retention time of the standard compound must be within 2% of the average retention time of that standard in the curve.

13.3.2. Initial calibrations of IS

13.3.2.1. Use the instrument software and specified parameters to perform peak integration for all identified peaks.

13.3.2.2. The IS is used for response and retention time reference. If there is an interfering component present in the sample that precludes accurate determination of the IS, the SUR (4-pentylphenyl 4-methylbenzoate) should be used as retention time reference.

13.3.2.3. Calculate the average retention time of the IS in the calibration curve and in each sample. The retention time of the IS in each sample must be within 2% of the average retention time of the standards in the curve.

13.3.2.4. Calculate the average response factor of the IS in the calibration curve and in each sample. The response factor for the IS must not vary by more than 10% from this average for each of the standards in the calibration curve.

Equation 5.

$$\text{Relative Retention Time} = \frac{RT_{STD}}{RT_{IS}}$$

Where RT_{STD} = Retention time of standard
RT_{IS} = Retention time of internal standard

13.3.3. QC and unknown samples

13.3.3.1. Apply the linear regression generated from the calibration standards to all QA/QC and unknown samples to calculate the concentration (µg/mL) of each cannabinoid using the instrument software or Excel spreadsheet. CCVs must not be used to calculate the concentration of analytes in samples.

13.3.3.2. **MS** and **MSD**

13.3.3.2.1. Calculate the % recovery of the MS and MSD.

13.3.3.2.2. To determine the precision, calculate the relative percent difference (RPD). The RPD must be <20%.

Equation 6.

$$\text{RPD} = \frac{|\text{MS} - \text{MSD}|}{\left(\frac{|\text{MS} + \text{MSD}|}{2}\right)} * 100$$

Where RPD is in percent (%).
MS = Matrix Spike concentration in ppb.
MSD = Matrix Spike Duplicate concentration in ppb.

13.3.3.3. Medical marijuana products

13.3.3.4. The final results for the marijuana products are reported as weight percentage (% C_S) using the following equations:

Equation 7.

$$C_s = \frac{C_X\left(\frac{\mu g}{mL}\right) * V_F(mL) * D}{M_I(mg) * 1000)}$$

Equation 8.

$$\% C_S = C_S * 100$$

Where C_S = Concentration of analyte in Sample (mass ratio)
$\% C_S$ = Concentration of analyte in Sample (%)
C_x = Concentration of analyte in Extract (µg/mL)
V_F = Final volume of extract (mL)
M_I = Initial mass of sample (mg)
D = Dilution factor, if applicable

13.4. Reporting of results

13.4.1. Non-detected analytes and analytes with a concentration <LOQ are reported as less than (<) the LOQ as specified in **Table 1.**

13.4.2. Analytes detected at a concentration at or above the LOQ are reported using 3 significant figures.

13.4.3. Total THC and total CBD are reported as mg/dose and calculated as follows:

Equation 9. Total THC = $(C_{delta\text{-}9\text{-}THC} + C_{delta\text{-}8\text{-}THC} + C_{THCA}) * M_{dose}$
Equation 10. Total CBD = $(C_{CBD} + C_{CBDA}) * M_{dose}$

C_{THC} = Concentration of THC calculated using equation 8.
$C_{THC\text{-}delta\ 9}$ = Concentration of THC delta-9 calculated using equation 8.
$C_{THC\text{-}delta\ 8}$ = Concentration of THC delta-8 calculated using equation 8.
C_{CBD} = Concentration of CBD calculated using from equation 8.
C_{CBDA} = Concentration of CBDA calculated using equation 8.
M_{dose} = Total mass of the dose (mg) calculated using equations 9 and 10.

13.4.4. For equations 9 and 10, where a component is less than the LOQ, the value of ‘0’ is substituted in the equation.

13.4.5. All results are reported through CLIMS.

14.0. Data Assessment, Acceptance Criteria and Corrective Actions for Out-of-Control Data

14.1. All analytical batches must meet all quality control criteria as described within this procedure and all QC results must be documented.

14.2. The acceptance criteria for standards and QC samples are defined in **Sections 10.0 and 11.0**. The sections below (**Sections 14.3 through 14.14**) outline the most common corrective action procedures for nonconforming data and inconsistent results. Since re-injection of a standard or sample is a routine corrective action for most nonconformities, it is not included in each individual section below, but may be used whenever applicable.

14.3. Failure to meet QC criteria for a calibration curve correlation coefficient of ≥0.995

14.3.1. Assess the calibration curve to determine if a standard appears to be prepared incorrectly.

14.3.2. If necessary, perform instrument maintenance.

14.3.3. A correlation factor of ≥ 0.995 must be achieved before sample analysis can begin. If samples were analyzed before an acceptable calibration curve was established, all affected samples must be re-analyzed under an acceptable curve or the results will be appropriately qualified.

14.4. Failure to meet required QC criteria for CCR of 85-115% recovery

14.4.1. A new CCR is prepared and re-analyzed. It may necessary to prepare it from a new working or stock solution.

14.4.2. If a newly prepared CCR still doesn't meet the required criteria, a new initial calibration curve is analyzed using existing or new calibration standards. The initial calibration curve is then verified with a CCR.

14.4.3. An acceptable CCR must be achieved prior to sample analysis. All samples associated with an unacceptable CCR must be reanalyzed.

14.5. Failure to meet required QC criteria for CCV of 90% to 110% recovery

14.5.1. A new CCV is prepared and analyzed. If necessary, prepare a new working or stock solution.

14.5.2. If a newly prepared CCV still doesn't meet the required criteria, a new initial calibration curve is analyzed using existing or new calibration standards. The initial calibration curve is then verified with a CCR.

14.5.3. All samples associated with an unacceptable CCV will be reanalyzed. If reanalysis in not possible due to lack of remaining extract or sample, the original sample results must be appropriately qualified.

14.6. Failure to meet required QC criteria for LOQ of 70 – 130% recovery

14.6.1. A new LOQ is prepared and re-analyzed. Prepare from a new working or stock solution, if necessary.

14.6.2. If a newly prepared LOQ still doesn't meet the required criteria, the instrument is recalibrated with new calibration standards, which may be prepared from new or existing working standard solutions or stock standard solutions. A new initial calibration curve is prepared, analyzed and verified with a CCR.

14.6.3. Acceptable LOQ recovery must be achieved before sample analysis can begin. If samples were analyzed before an acceptable LOQ was achieved, all affected samples must be re-analyzed after an acceptable LOQ is achieved.

14.7. Failure to meet required QC criteria for SBLK of <1/3 LOQ for target analyte(s) in routine sample batches

14.7.1. Change the HPLC column.

14.7.2. Inject SBLK and run through the system until background contamination is removed or reduced to an acceptable level.

14.7.3. An acceptable SBLK must be achieved before sample analysis begins. If samples have already been analyzed, then any samples containing target analytes must be re-analyzed. If re-analysis of suspect samples is not possible due to lack of remaining extract or sample, the original sample results will be appropriately qualified.

Exception - If the samples do not contain target analytes at or above the LOQ, the original results may be reported without re-analysis and qualification is not necessary.

14.8. Failure to meet required QC criteria for MB of <1/3 LOQ for target analyte(s)

14.8.1. The source of contamination shall be investigated, and measures taken to minimize or eliminate the problem. Affected samples will be reprocessed or data shall be appropriately qualified if:

a) the concentration of a targeted analyte in the blank is at or above the reporting limit as established by the method or by regulation, AND is greater than 1/10 of the amount measured in the sample;
b) the blank contamination otherwise affects the sample results as per the method requirements or the individual project data quality objectives; and
c) a blank is determined to be contaminated. The cause shall be investigated, and measures taken to minimize or eliminate the problem. Samples associated with a contaminated blank shall be evaluated as to the best corrective action for the samples (e.g., reprocessing or data qualifying codes). In all cases, the corrective action shall be documented.

14.9. LCS with <80% or >120% recovery

14.9.1. See Section 14.10.

14.10. MS and MSD with <80% and >120% recovery or RPD >20%

14.10.1. If the MS and/or MSD fails to meet the acceptance criteria for any target analytes, it is recommended that the MS and/or MSD be re-prepared and analyzed if sufficient sample remains; this may require preparation from a new stock standard.

14.10.2. Re-analysis is not required; however, if the MS and/or MSD fails with high recovery and no target analytes are detected in the batch, as LOQ sensitivity is shown, or if all other quality control measures within the batch are acceptable.

14.10.3. If re-analysis is performed and the new MS and/or MSD meets the acceptance criteria, only report the results from the re-analysis.

14.10.4. The relative percent difference (RPD) for each spiked analyte in the MS and MSD must be <20%.

14.10.5. If the new MS and/or MSD still fails, the original MS and/or MSD and corresponding sample results must be appropriately qualified.

Exception – if the MS and/or MSD fails with high recovery and no target analytes are detected in the batch, qualification is not necessary as LOQ sensitivity is shown.

14.11. IS area in sample deviates by > 10% from area in most recent CCV

14.11.1. Re-inject the sample extract.

14.11.1.1. If the results of the re-injection meet acceptance criteria, only report the results of the re-injection.

14.11.1.2. If re-injection still fails, re-extract and re-analyze the sample.

14.11.1.3. If additional sample is not available, results must be reported with appropriate qualifiers.

14.11.2. In the event of an interference(s) (causing >10% error) with the internal standard.

14.11.2.1. Cannabinoid concentrations are calculated using an external calibration.

14.11.2.2. Cannabinoid concentrations in the QC samples should be calculated the same way as the other unknown samples without using the internal standard correction.

14.11.2.3. When the internal standard correction is not used in determining the cannabinoid concentrations, all sample analyses must be finished within 48 h of sample preparation to avoid significant error caused by evaporation of solvent from the samples.

14.11.2.4. If all quality assurance criteria are met in **Section 11.0**, samples are reported with the appropriate qualifiers.

14.12. SUR with <80% or >120% recovery

14.12.1. Re-prepare the sample and analyze if a duplicate sample remains.

14.12.2. If the re-analysis meets acceptance criteria, only report those results.

14.12.3. If the re-analysis still fails, the original sample results will be appropriately qualified.

Exception – if the SUR fails with high recovery and no target analytes are detected in the sample, qualification is not necessary as LOQ sensitivity is shown.

14.13. Inconsistent baseline

14.13.1. Perform appropriate instrument maintenance, if applicable.

14.13.2. Repeat the sequence using the same standards/samples. If repeat analysis is acceptable, report only the analytical results from the repeated analysis.

14.13.3. If instrument maintenance and repeat analysis fails to produce acceptable data, the sample results will be appropriately qualified.

14.14. All other nonconforming data, not addressed within this procedure, requires the completion of a nonconformance/corrective action report.

15.0. Method Performance

15.1. LOD and DOC study results are maintained by the laboratory.

15.2. Preventative Maintenance is performed on the instrument once per year. This maintenance pertains to the lamp, pumps and data system.

16.0. Waste Management/Pollution Prevention

16.1. It is the responsibility of the laboratory to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions.

16.2. Minimize solvent, chemical, reagent and standard use whenever possible to reduce the amount of hazardous waste generated.

16.3. Dispose of solvent waste in an appropriate solvent waste container (red, 5-gal solvent can), properly labeled (separate chlorinated and non-chlorinated solvents).

16.4. Dispose of non-hazardous aqueous waste in the laboratory sink followed by flushing with tap water.

16.5. Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal

17.0. References

17.1. Definition and Procedure for the Determination of the Method Detection Limit, Environmental Protection Agency, 40 CFR Part 136, Appendix B.

17.2. Shimadzu LabSolutions "LC Getting Started Guide" http://www.shimadzu.com/an/data-net/labsolutions/labsol-2.html

17.3. Public Health Law, section 502 of the Public Health Law (PHL), Title 10 (Health) of The Official Compilation of Codes, Rules and Regulations of the State of New York (NYCRR) subpart 55-2 (Approval of Laboratories Performing Environmental Analysis). http://w3.health.state.ny.us/dbspace/NYCRR10.nsf/56cf2e25d626f9f785256538006c3ed7/c9252587bc832b3485256c390055920a?OpenDocument&Highlight=0,section,55)

17.4. *Norgestrel*; MSDS No. N2260 [Online]; Sigma-Aldrich: Saint Louis MO, September 03, 2014 http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do

18.0. Supporting Documents

18.1. Measurement of Phytocannabinoids in Medical Marijuana using HPLC-PDA (MML-300-AppA).
18.2. Medical marijuana sample preparation protocols for potency analysis (MML-301-SOP)

19.0. Appendices

Appendix A - MML-300-AppA

Figure 1- 5 µg/mL ISTD in blank solvent

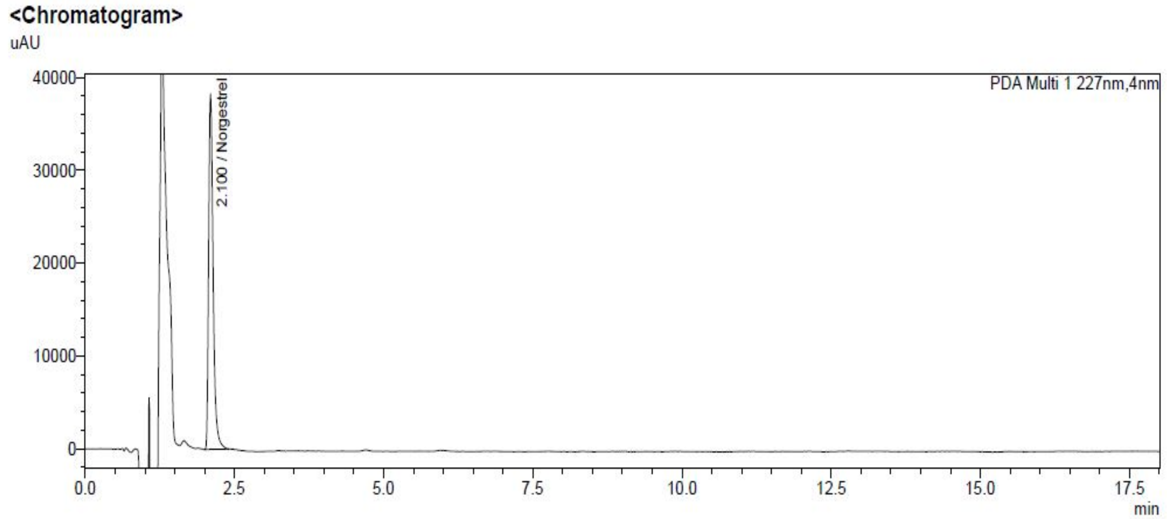
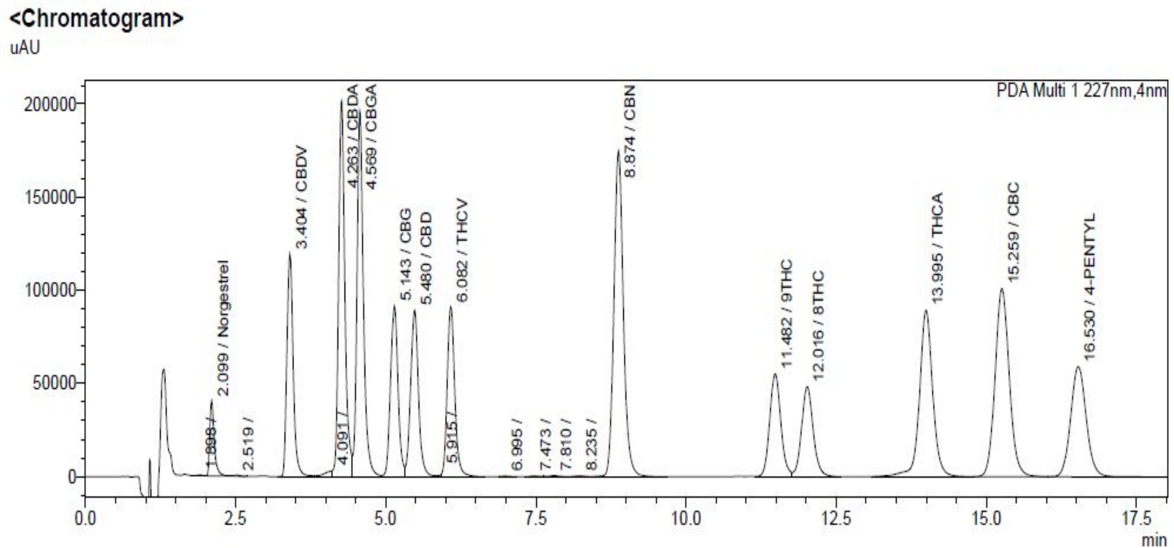


Figure 2- 45.0 µg/ml w/ ISTD and Surrogate



Appendix B

Table 1 – HPLC-PDA

Cannabinoids, Internal Standard and Surrogate with corresponding retention times.

Analyte	Full name	Nickname	Retention time*
1.	Norgestrel	ISTD	2.09
2.	Cannabidivarin	CBDV	3.40
3.	Cannabidiolic Acid	CBDA	4.26
4.	Cannabigerolic Acid	CBGA	4.57
5.	Cannabigerol	CBG	5.14
6.	Cannabidiol	CBD	5.48
7.	Tetrahydrocannabivarin	THCV	6.06
8.	Cannabinol	CBN	8.87
9.	Delta-9 Tetrahydrocannabinol	THC-9	11.48
10.	Delta-8 Tetrahydrocannabinol	THC-8	12.02
11.	Tetrahydrocannabinolic Acid	THCA	14.00
12.	Cannabichromene	CBC	15.26
13.	4- pentylphenyl 4-methylbenzoate	Surrogate	16.53

*Retention Time acceptable within 2%
Retention times are approximate based on current column setup and may vary slightly over different column installations and the lifetime of the columns.
Columns and analytical conditions are described in Section 9.

U.S. FOOD & DRUG ADMINISTRATION

Memorandum

To: Foods Program Governance Board
From: FDA Foods Program Regulatory Science Steering Committee (RSSC)
Date: October 17, 2019
Subject: Guidelines for the Validation of Chemical Methods for the FDA Foods Program, 3rd Edition

The FDA Foods Program Regulatory Science Steering Committee (RSSC), made up of representatives from the Center for Food Safety and Applied Nutrition (CFSAN), the Center for Veterinary Medicine (CVM), the Office of Regulatory Affairs (ORA), the National Center for Toxicological Research (NCTR), and the Office of the Chief Scientist of the FDA, is charged with the task of prioritizing, coordinating and integrating human food- and animal food-related science and research activities across the operating units of FDA's Foods Program.

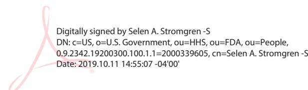
As a regulatory agency tasked with ensuring the safety of the nation's food supply, it is imperative that the laboratory methods needed to support regulatory compliance, investigations and enforcement actions meet the highest analytical performance standards appropriate for their intended purposes. Development of standardized validation requirements for all regulatory methods used in our laboratories to detect chemical and radiological contaminants, as well as microbial pathogens, is a critical step in ensuring that we continue to meet the highest standards possible.

The attached document, now formally adopted by the RSSC, updates and renews the requirements that must be fulfilled in the evaluation of chemical methods to be used in our testing laboratories and supersedes the prior guidelines. These updated guidelines are posted on FDA's Foods Program Methods website. Please share these chemical methods validation guidelines with anyone who may be conducting or supervising chemical methods validation projects or otherwise needs to be aware of these updated requirements.

As one of the hierarchical committees under the RSSC, the Chemical Methods Validation Subcommittee (CMVS) is charged with providing guidance and oversight to all validation studies and is principally responsible for the content of these Guidelines, with input from the Chemistry Research Coordination Group (CRCG) and associated Technical Advisory Groups. Additional questions and comments about the Guidelines may be directed to the CMVS or CRCG.

Thank you,

Selen A.
Stromgren -S



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Selen Stromgren, Ph.D., Chair RSSC

Guidelines for the Validation of Chemical Methods in Food, Feed, Cosmetics, and Veterinary Products

3rd Edition

U.S. Food and Drug Administration

Foods Program

October 2019

ACKNOWLEDGMENT

The third edition of these guidelines was published in 2019 at the request of the US FDA Foods Program. In cooperation with members of the Foods Program Regulatory Science Steering Committee, direct input, review, and consent were provided by the following FDA research and regulatory offices:

Center for Food Safety and Applied Nutrition
Office of Regulatory Science
Office of Food Safety
Office of Applied Research and Safety Assessment

Center for Veterinary Medicine
Office of Research
Office of New Animal Drug Evaluation

Office of Regulatory Affairs
Office of Regulatory Science

APPROVAL PAGE

This document is approved by the FDA Foods and Veterinary Medicine (FVM) Regulatory Science Steering Committee (RSSC). RSSC is the new committee that replaces the Science and Research Steering Committee. The **FVM RSSC Project Manager is responsible for updating the document as change requirements are met, and disseminating updates to the RSSC and other stakeholders, as required.**

APPROVED BY:

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RSSC Co-chair

US Food & Drug Administration
Office of Foods and Veterinary Medicine

Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd *Edition*

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1.0 INTRODUCTION

1.1 Purpose

The U.S. Food and Drug Administration (FDA) is responsible for ensuring the safety of approximately 80% of the nation's food supply. FDA laboratories contribute to this mission through routine surveillance programs, targeted regulatory analyses, and emergency response when contaminated food or feed is detected or suspected in a public health incident. The effectiveness of these activities is highly dependent on the quality and performance of the laboratory methods needed to support regulatory compliance, investigations and enforcement actions. To ensure that the chemical methods employed for the analysis of foods and feeds meet the highest analytical performance standards appropriate for their intended purposes the Regulatory Science Steering Committee (SRSC) has established criteria by which all Foods and Veterinary Medicine (FVM) Program chemical methods shall be evaluated and validated. This document defines four standard levels of performance for use in the validation of analytical regulatory methods for chemical analytes in foods and feeds.

1.2 Scope

These criteria apply to FDA laboratories as they develop and participate in the validation of analytical regulatory methods for chemical analytes in food, feed, and cosmetics in anticipation of Agency-wide FVM Program implementation. These criteria do not apply to methods developed by or submitted to FDA under a codified process or official guidance (e.g., in the Code of Federal Regulations, CPGs, etc.), such as for veterinary drug approval. For such studies, the appropriate Center for Veterinary Medicine (CVM) or other Program guidance documents should be followed. This guidance is a forward-looking document; the requirements described here will only apply to *newly*-developed methods and significant modifications to existing methods (see Requirements). Once a method has been validated at the appropriate level, it can be implemented according to document, FDA-OFVM-3, "Methods Development, Validation, and Implementation Program," which establishes a standard operating procedure for the methods development, validation and implementation process [1]. For example, a multi-laboratory validated method to be used in a widespread regulatory application can be implemented by other FDA laboratories following the method verification process.

1.3 Administrative Authority and Responsibilities

All criteria established in this document for analytical method validation have been adopted and approved by the RSSC. The document, FDA-OFVM-3, establishes the standard operating procedure for the approval and tracking of method development and validation activities within the FVM Program [1]. Single laboratory validation (SLV) studies (including both Level 1 and Level 2 validations) can be managed wholly by the respective Center and Office line management structure. Oversight and coordination of multi-laboratory validation (MLV) studies (including both Level 3 and Level 4 validations) are the responsibility of the Methods Validation Subcommittees (MVS).

1.4 The Method Validation Subcommittee

Under the charge of the RSSC, the Chemistry Methods Validation Subcommittee (CMVS) will have oversight responsibility for MLV studies involving chemical methods associated

with the FVM Program which are intended for use in a regulatory context. The CMVS is a subcommittee of the Chemistry Research Coordinating Group (CRCG), which reports directly to the RSSC. The CMVS is governed by the organizational structure, roles and responsibilities as detailed in its charter [2]. Briefly, the CMVS will oversee and coordinate, in collaboration with the originating laboratory, all MLV studies for chemical methods developed within the FDA FVM Program to support regulatory analytical needs. This includes the evaluation and prioritization of proposed MLV studies as well as evaluation of completed MLV studies and reports. Submissions of chemical validation proposals, reports, questions, *etc.* can be directed to the CMVS through a central email account:

Chemistry.mvs@fda.hhs.gov

However, where possible, MLVs should be discussed in appropriate Technical Advisory Groups or with the CRCG to ensure the broadest possible consideration of factors before committing resources to an MLV.

1.5 General Responsibility of the Originating Laboratory

It is the responsibility of the originating laboratory to ensure proper adherence to all criteria described in this document. The originating laboratory should work in consultation with the CMVS and/or its designated Technical Advisory Group (TAG) throughout the multi-laboratory validation process. It will be the responsibility of the originating laboratory to include their respective QA/QC manager in all aspects of the validation process.

1.6 Overview of Method Validation

Method validation is the process of demonstrating or confirming that a method is suitable for its intended purpose. The purpose of these methods may include but is not limited to qualitative analysis, quantitative analysis, screening analysis, confirmatory analysis, limit tests, matrix extensions, platform extensions, and emergency/contingency operations. Validation includes demonstrating performance characteristics such as accuracy, precision, sensitivity, selectivity, limit of detection, limit of quantitation, linearity, range, and ruggedness, to ensure that results are meaningful.

Method validation is a distinct phase from method development/optimization and should be performed *subsequent* to method development. Methods may be validated for one or more analytes, one or more matrices, and one or more instruments or platforms. The method is validated by conducting experiments to determine the specific performance characteristics that serve to define and quantify method performance.

1.7 Applicability

This document establishes validation criteria for regulatory methods that are to be widely used to detect and quantitate chemical analytes in food, feed and other FDA regulated products covered by the FVM Program including, but not limited to, the following:

Chemotherapeutic Residues
Color Additives
Decomposition Products
Dietary Supplement Ingredients/Adulterants
Elemental and Metals
Food and Feed Additives and Preservatives
Food Allergens
Gluten

Intentional Adulterants/Poisons
Mycotoxins
Nutrients
Persistent Organic Pollutants
Pesticides
Seafood and plant toxins
Toxic Elements
Veterinary Drug Residues

1.8 Requirements

Method validation is required for:

- Submission of a new or original method.
- Expansion of the scope of an existing method to include additional analytes.
- Expansion of the scope of an existing method to include additional matrices.
- Changes in the intended use of an existing method (*e.g.,* screening vs. confirmatory).
- Modifications to a method that may alter its performance specifications (*e.g.*, modifications that could significantly affect the precision and accuracy, changes to the fundamental science of an existing method, significant changes to reagents, apparatus, instrumental parameters, sample preparation and/or extraction, or modification of a method's range beyond validated levels). Allowable modifications that would not require further validation are provided in Appendix 6 for Mass Spectrometry (GC and LC) methods and in the document ORA-LAB.5.4.5 Attachment A-Modification Criteria [3] for HPLC and GC (non-MS) methods.

2.0 CRITERIA AND GUIDANCE FOR THE VALIDATION OF CHEMICAL METHODS

2.1 General Validation Tools and Protocol Guidance

There are a number of excellent references and guides available providing further information on method validation for chemical methods [3-20]. The following provides some general guidelines/tools that should be used to assess method performance:

General Protocol: Prepare and analyze method blanks, matrix blanks, reference materials (if available) and matrix spikes (using matrix blanks if available) of known concentration as generally described under the Methods Validation Levels section and Table 1 below. Accuracy or bias and precision are calculated from these results. Data will also be used to evaluate matrix effects and ruggedness/robustness of the method resulting from changes in the sample matrix.

The following general validation tools should be used to generate method performance characteristics as described in the Performance Characteristics section below.

Blanks: Use of various types of blanks enables assessment of how much of the result is attributable to the analyte in relation to other sources. Blanks are useful in the determination of limit of detection.

Reference materials and certified reference materials: The use of known reference materials (when available and applicable) should be incorporated to assess the accuracy or bias of the method, as well as for obtaining information on interferences.

Matrix Blank: This type of blank is a substance that closely matches the samples being analyzed with regard to matrix components. Matrix blanks are used to establish background level (presence or absence) of analyte(s) and to verify that sample matrix and equipment used does not interfere with or affect the analytical signal.

Matrix Spikes (Laboratory Fortified Matrix): Recovery determinations can be estimated from fortification or spiking with a known amount of analyte and calculation of spike recoveries. (Note: spike recovery may not be accurately representative of recovery from naturally incurred analytes.) Matrix effects can also be assessed with these samples. Accuracy or bias and precision are calculated from these results. The data can also be used to evaluate robustness of the method resulting from changes in the sample matrix.

Incurred Samples: This type of sample contains (not laboratory fortified) the analyte(s) of interest (if available) and can be used to evaluate precision and bias (if analyte concentration(s) are reliably known). Analyte recovery can also be evaluated through successive extractions of the sample and/or comparison to another analytical procedure with known bias.

Reagent Blank: This type of blank incorporates all reagents used in the method and is subjected to all sample processing operations. It serves to verify that reagents are analyte free and the equipment used does not interfere with or affect the analytical signal.

Replicate Analyses: The precision of the analytical process can be evaluated using replicate analyses. The originating laboratory should assure that adequate sample replicates are

performed and that results from replicate measurements of each analyte are compared. Minimally, the method repeatability should be evaluated.

Interferences: Spectral, physical, and chemical interferences can be evaluated by analyzing samples containing various suspected interferences. Carryover should be evaluated using the incorporation of blanks immediately following standards and samples.

Statistics: Statistical techniques are employed to evaluate accuracy, trueness (or bias) precision, linear range, limits of detection and quantitation, and measurement uncertainty.

2.2 Reference Method

A reference method is a method by which the performance of an alternate or new method may be measured or evaluated. For chemical analytes, an appropriate reference method is not always identifiable or available. However, there are some instances in which the use of a reference method is appropriate such as when replacing a method specified for use in a compliance program. Consultation between the originating laboratory and the CMVS and the Program Office is suggested when deciding if the use of a reference method will be necessary.

2.3 Performance Characteristics

Performance characteristics that should be evaluated in order to validate a method will vary depending on the intended use of the method, the type of method (*e.g.*, quantitative vs. qualitative), and the degree to which it has been previously validated (e.g., matrix extension, analyte extension, platform extension). Although definitions of these characteristics are included in Appendix 1, this document is not meant to address the various ways of calculating characteristics such as method detection level, limit of detection or limit of quantitation.

Performance Characteristics for Validation of New Quantitative Methods: Validation of new quantitative methods should include at a minimum evaluation of the following performance characteristics: accuracy, precision, selectivity, limit of detection, limit of quantitation, linearity (or other calibration model), range, measurement uncertainty, ruggedness, confirmation of identity and spike recovery.

Performance Characteristics for Validation of New Qualitative Methods: Validation of new qualitative methods should include at a minimum evaluation of the following performance characteristics: sensitivity, selectivity, false positive rate, false negative rate (guidance for determining false positive/negative rates is in Appendix 2B), minimum detectable concentration, ruggedness, and confirmation of identity.

Performance Characteristics for Validation of Method Extensions: Validating the extension of methods that have previously been validated requires a careful evaluation of the intended purpose of the extension. In cases where the sample preparation and/or the extraction procedure/analytical method is modified from the existing test procedure, it should be demonstrated that the modifications do not adversely affect the precision and accuracy of the data obtained. In order to implement the modified method, generally the standard or existing method is first performed. The modified method performance then is verified by comparison with that of the original method.

2.4 Confirmation of Identity

Confirmation of identity for each analyte must be performed as part of the method validation for regulatory enforcement for both qualitative and quantitative methods. Unambiguous confirmation of identity usually requires analytically identifying key features of each analyte in the scope of the new method being validated such as with mass spectral fragmentation patterns or by demonstration of results in agreement with those obtained using an independent analysis.

FDA has issued guidance documents on the development, evaluation, and application of mass spectrometric methods for confirming the identity of target analytes including: CVM Guidance for Industry 118: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues [4] and Acceptance Criteria for Confirmation of Identity of Chemical Residues using Exact Mass Data within the Office of Foods and Veterinary Medicine [5] Following the CVM guidance is required for veterinary drug residue methods. For other types of chemical contaminants in food (e.g. food additives, mycotoxins, etc.), the CVM document should be followed because it was written as a Guidance for Industry and therefore has been more widely internally and externally reviewed and distributed.

2.5 Method Validation Levels

The following describes the four standard levels of performance defined for method validation of analytical regulatory methods for chemical analytes in foods. This approach is based on the Food Emergency Response Network (FERN), SOP No: FERN-ADM.0008.00, FERN Validation Guidelines for FERN Chemical, Microbiological, and Radiological Methods [6], as well as AOAC guidelines for single laboratory validation [7] and collaborative studies [8]. Key validation parameters for each level are summarized in Table 1. It is the responsibility of the originating (developing) laboratory to determine the appropriate level of validation required up to and through single laboratory validations. It is highly recommended that originating laboratories work with the appropriate Technical Advisory Group when determining the appropriate level of validation.

NOTE: *Not all methods will or should be validated to the highest level.*

Level One

This is a single laboratory validation level with the lowest level of validation requirements and is appropriate for emergency/limited use. Performance of the method at this initial level of scrutiny will determine, in part, whether further validation is useful or warranted.

Intended Use: emergency/limited use/matrix extension/analyte extension/platform extension. Examples of where Level One validation would be acceptable include isolated consumer complaints, single-occurrence samples, and application of a method developed for a specific analyte(s) to a matrix not previously validated, in response to a real or perceived threat to food safety or public health. Validation of method performance with a new matrix is intended to assure that the new matrix will produce accurate and reliable results for all the analytes in the scope of the method. Generally, all targeted analytes still must be included in matrix spikes at this level, if widespread use in this matrix is anticipated for regulatory purposes. As the first level of validation of methods for matrix, analyte or platform extension/emergency use, it would be expected that a more rigorous single laboratory validation at least equivalent to Level Two below would be performed before more widespread non-emergency regulatory use. For further guidance on extensions, see Appendix 5.

Level Two

This is a single laboratory validation level. The originating lab has conducted a comprehensive validation study, with performance criteria similar to an AOAC Single

Laboratory Validation study. If appropriate, a comparison with an existing reference method has been performed. Some of the criteria of the study may be at a lower level than the AOAC Single Laboratory Validation study,but are appropriate for the developing method at this stage.

Intended Use: Routine regulatory testing, emergency needs, minor method modifications, analyte and matrix extensions of screening methods. If a method validated at this level is expected to have use that is widespread, long term, of high public visibility or potentially involved in international trade conflicts, its validation should be extended to at least Level Three below.

Level Three

This is a multi-laboratory validation level. Level Three validation employs a minimum of one collaborating laboratory in addition to the originating laboratory. Most of the criteria followed by the originating lab are at a level similar to the AOAC full collaborative study level with comparison to an existing reference method when available and appropriate. The additional collaborating laboratories follow many of the criteria found in an AOAC collaborative study. The main differences are that Level Three validation employs at least one additional collaborating laboratory instead of the eight to ten used by AOAC and requires fewer replicates for each food matrix/spike level. MLV's are studies of the method, not the laboratory. The method must be followed as closely as practicable, and any deviations by participants from the method described, no matter how trivial they may seem, must be noted on the report form [8].

Intended Use: Methods validated to this level of scrutiny are acceptable for use in all regulatory circumstances including screening analyses, confirmatory analyses, regulatory surveys, and compliance support. If the method is expected to have use that is widespread, long term, of high public visibility or involved in international trade conflicts, it may be appropriate to have its validation extended to Level Four.

Level Four

This validation level has criteria equivalent to a full AOAC or ISO Collaborative Study. Any method reaching this level of validation should be able to be submitted for adoption by the AOAC as a fully collaborated method. MLV's are studies of the method, not the laboratory. The method must be followed as closely as practicable, and any deviations by participants from the method described, no matter how trivial they may seem, must be noted on the report form [8].

2.6 Acceptability Criteria

There are various acceptability ranges for method validation performance criteria that may be appropriate depending on the application or intended use of the methodology and especially the levels of concern, action levels or tolerance for the chemical analyte. Some examples of acceptability ranges used by various national and international organizations and their sources are provided in Appendix 2. Acceptable spike recoveries vary with analyte concentration as indicated in Appendix 2 (e.g., recoveries may fall in approximately the 80- 120% range for quantitative methods at the 1 µg/g (ppm) concentration). Repeatability and reproducibility also vary with analyte concentration. The acceptability ranges in Appendix 2 provide approximate target ranges for method developers and the MVS and are not rigid binding guidelines. It is recognized that for some situations such as with difficult matrices, extremely low analyte concentrations (e.g., chlorinated dioxins, persistent organic pollutants), multi-residue methods and with emergency situations these general acceptability ranges may not be achievable or required.

Table 1. Key Validation Parameter Requirements for Chemical Methods

	Level One: Emergency/ Limited Use	Level Two: Single Laboratory Validation	Level Three: Multi-Laboratory Validation	Level Four: Full Collaborative Study
Number participating labs	1	1	≥ 2	8 (quantitative) 10 (qualitative)
Number of matrices*	≥1	≥3 recommended where available	≥3 recommended where available	≥3 recommended where available
Number of analyte(s) spike levels for at least one matrix source**	≥2 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank	≥3 spike levels + 1 matrix blank
Replicates required per matrix source at each level tested per laboratory	≥2 (quantitative) ≥2 (qualitative)	≥2 (quantitative) ≥3 (qualitative)	≥2 (quantitative) ≥3 (qualitative)	≥2 (quantitative) ≥3 (qualitative)
Replicates required at each level tested per laboratory if only one matrix source used	≥4 (quantitative) ≥6 (qualitative)	≥6 (quantitative) ≥9 (qualitative)	≥3 (quantitative) ≥6 (qualitative)	≥2 (quantitative) ≥6 (qualitative)

*If a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more, but if only one food matrix is studied then ≥3 sources are recommended, where available. The number of matrix sources may be reduced, particularly if it is difficult to obtain blank matrix sources, as long as the total number of spike levels and matrix combinations are adequate (e.g., 6 replicates or greater at each spike level for quantitative methods and 9 replicates or greater for qualitative methods). Certified reference materials/ incurred tissues should be used, when available, and can replace one of your spiking levels.
** Number of spike levels is recommended for at least one source of matrix. Other similar sources of matrix (*e.g.,* within the same category; see Appendix 4) may be studied at one or two spike levels (*e.g.*, at an action/guidance or tolerance level or close to the lower limit of quantitation/detection). Certified reference materials/ incurred tissues should be used, when available, and can replace one of your spiking levels. For some analytes, spiking with pure standard alone does not sufficiently demonstrate method performance (i.e. BPA in can coatings contain oligomeric interferences, gluten in fermented/hydrolyzed products, protein-bound veterinary drug metabolites, sulfites binding irreversibly after spiking). In these cases, reference materials and/or real samples must be used to demonstrate method performance.

3.0 ADDITIONAL PROCEDURAL GUIDANCE

In addition to the criteria described above in Table 1 for standard quantitative and qualitative methods, additional guidance is provided in this section for specific types of methods or validation situations.

3.1 Platform/Instrumentation Extension

Expanding the use of a validated method to include another significantly different instrument or platform requires further validation. Such instances include the use of an instrument or platform similar in scope and function to that currently validated and approved for use; however, it may have major differences in configuration, or detection scheme. Detailed guidance for platform extensions are in Appendix 5.

3.2 Analyte Extension

Multi-residue, multi-class methods are becoming more common. Many of these methods are semi-quantitative (limits tests) or qualitative broad band screens. Performance requirements for these types of procedures are described below. However, if a multi-residue method is meant to be used for quantitation, the same performance characteristics as required for single analyte methods should be evaluated for each analyte (accuracy, precision, selectivity, limit of detection, limit of quantitation, linearity range, uncertainty, and ruggedness). It is understood that with a large multi-residue method, not all analytes will meet the recommended acceptability ranges listed in Appendix 2, but the performance for each compound should be tested and reported so that the accuracy and precision are known for any given analyte and are sufficient for the intended purpose of the method. Detailed guidance for validation required for analyte extensions is described in Appendix 5.

3.3 Food Matrix Extension

The validation of method performance with a new matrix is intended to assure that the method will continue to produce accurate and reliable results. It is generally assumed that the more closely related a new food matrix is to a previously validated matrix for a defined analyte, the greater the probability that the new matrix will behave similarly. It is also usually the case that the regulatory chemical methods employed by FDA are used to analyze a diversity of products representing a large spectrum of matrices. Detailed guidance for matrix extensions is in Appendix 5.

3.4 Limit Tests (common semi-quantitative screening method)

One specific category of qualitative methods includes limit tests (binary or pass/fail tests) for analytes that have a defined level of concern. The purpose of these screening methods is to determine if analyte is present with a concentration near or above the level of concern. This is in contrast to screening methods whose intended purpose is to determine the presence or absence of an analyte at any level. Limit test method validations must include determination of the precision of the method for an analyte(s) at the level(s) of concern.

Limit test screening methods, in general, should avoid false negatives with false negative rates representing less than 5% of the analytical results (see Appendix 2B for determining false positive/negative rates). The occurrence of false positives is less critical since presumptive positives are further analyzed by quantitative or confirmatory

methods. However, false positive rates should typically be less than 10-15% to avoid unnecessary confirmatory testing. Ideally, limit tests are capable of rapidly screening a large number of samples to minimize the need for additional analysis. A common approach used in limit test screening methods is to use a confidence interval to set a laboratory threshold or cut-off value whereby only responses above that value require further testing. For a limit

test based on an instrument response, a threshold or cut-off value can be determined by a confidence limit, based on an estimate of the standard deviation of the response or concentration of an analyte in samples fortified with the analyte at the level of concern.

Example:
Milk samples (n=21) were fortified with sulfamethazine at the level of concern (10 ng/mL). A LC-MS/MS limit test screening method was used to measure this drug in the extracted milk samples. The mean concentration found was to be 10.99 ng/mL with a standard deviation of 2.19. A threshold or cut-off value was calculated so that 95% of samples containing sulfamethazine at or above 10 ng/mL would have a response above the threshold value:

Threshold value = [mean concentration – (t * standard deviation)]
= [10.99 – (1.725 * 2.19)] = 7.21 ng/mL

Where t = one-tailed Student's t value for n-1 degrees of freedom at the 95% confidence level

This approach can also be used for immunosorbent assays such as enzyme linked immunosorbent assay (ELISA) or optical biosensor assays. These tests may be non-competitive (direct measurement of analyte response) or competitive (indirect measurement). Analysis of data from a competitive immunosorbent test should account for the fact that the observed response decreases with increasing analyte concentration; therefore, a response lower than the threshold or cut-off would be considered a presumptive positive response. For immunosorbent assays, it is also important to measure the response observed for blank matrix samples and to verify that the blank response is distinguishably (statistically) different from that of the threshold.

Performance characteristics of limit tests:
Validation of new limit tests should include, at a minimum, evaluation of the following performance characteristics: sensitivity, specificity, precision, threshold or cut-off value, false positive rate, false negative rate, minimum detectable concentration (should be lower than the threshold/cut-off value), and ruggedness/robustness.

3.5 Qualitative Broad-band Analyte Screening

Broad-band methods that can detect many compounds are being utilized more frequently as an initial screening step as part of chemical contaminant testing in FDA laboratories. These methods usually involve mass spectrometric analyses and provide qualitative information. For example, the data obtained may be compared to an established reference such as a database of compounds with exact mass and molecular formula information or spectra in a compiled library.

Typically, initial validation of these methods is performed using a limited set of representative analytes and representative matrices. For example, sets of analytes that contain compounds from a variety of chemical classes from the area of interest (e.g. pesticides,

veterinary drug residues, or common chemical toxins) are tested with the method using representative matrices. The performance characteristics that may be evaluated include: sensitivity, selectivity, false positive rate, false negative rate (see Appendix 2B for calculation of false positive/negative rates), minimum detectable concentration, ruggedness, and confirmation of identity. It is understood that the method performance may vary with the different classes of compounds, but it is important to have an initial evaluation of the method's capabilities.

Laboratories continuously expand the scope of these broad-band methods by adding new analytes that come to their attention through various sources of intelligence. In addition, a new compound might be found in a sample after acquired data are compared to the reference databases. In these cases, some verification that the analyte can be detected reliably by the screening method is required. When a new compound is added to the scope of a qualitative method, it should first be determined whether this compound belongs to a class of compounds that has already been validated for the broad-band method. If the new compound shares chemical characteristics with an existing class of compounds in the scope of the method, then it may suffice to select a few representative matrices, perform a single level spike in these representative matrices in duplicate and determine that reproducible recovery is obtained in order to assess whether the analyte can be detected effectively by the method. Scenarios that may require a full validation would include a new analyte being added to the scope of the broad-band method that was not represented by any of the compound classes already in the scope. Also, if the new analyte requires modifications in the extraction protocol due to its chemical characteristics, then its inclusion in the scope should be fully validated as recommended by this guidance.

Although positive findings by the broad-band method are subjected to confirmatory testing using a targeted method, it is still important to determine, through proper validation and verification protocols, that the broad-band method does not give rise to a high number of false negative findings. False negative in this context means the method fails to detect a residue in its scope when the residue is present in the matrix at or above the level of concern or minimum detectable concentration. While the positive finding by the broad-band method is subjected to further analysis and scrutiny, negative findings are upheld as such and a regulatory decision is made based on these results, *e.g.*, to release the products into commerce.

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APPENDIX 1 - Glossary of Terms

Generally, references 13-17 were utilized in preparation of this glossary.

Accuracy: The closeness of agreement between a test result and an accepted reference value. When applied to test results, accuracy includes a combination of random and systematic error. When applied to test method, accuracy refers to a combination of trueness and precision.

Action level: Level of concern or target level for an analyte that must be reliably identified or quantified in a sample.

Analyte: The chemical substance measured and/or identified in a test sample by the method of analysis.

Analytical batch: An analytical batch consists of samples, standards, and blanks which are analyzed together with the same method sequence and same lots of reagents and with the manipulations common to each sample within the same time period (usually within one day) or in continuous sequential time periods.

Bias: The difference between the expectation of the test result and the true value or accepted reference value. Bias is the total systematic error, and there may be one or more systematic error components contributing to the bias.

Blank: A substance that does not contain the analytes of interest and is subjected to the usual measurement process. Blanks can be further classified as method blanks, matrix blanks, reagent blanks, instrument blanks, and field blanks.

Calibration: Determination of the relationship between the observed analyte signal generated by the measuring/detection system and the quantity of analyte present in the sample measured. Typically, this is accomplished through the use of calibration standards containing known amounts of analyte.

Calibration Standard: A known amount or concentration of analyte used to calibrate the measuring/detection system. May be matrix matched for specific sample matrices.

Carryover: Residual analyte from a previous sample or standard which is retained in the analytical system and measured in subsequent samples. Also called *memory*.

Certified Reference Material (CRM): Reference material accompanied by documentation (certificate) issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures. Note: Standard Reference Material (SRM) is the trademark name of CRMs produced and distributed by the National Institute of Standards and Technology (NIST).

Check Analysis: Result from a second independent analysis which is compared with the result from the initial analysis. Typically, check analyses are performed by a different analyst using the same method.

Confirmation of Identity: Unambiguous identification of an analyte(s) by a highly specific technique such as mass spectrometry or by demonstration of results from two or more independent analyses in agreement.

Confirmatory Analysis/Method: Independent analysis/method used to confirm the result from an initial or screening analysis. A different method is often used in confirmation of screening results.

Cut-off Concentration: In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods). See also *Threshold Value*.

False Negative Rate: In qualitative analysis, a measure of how often a test result indicates that an analyte is not present, when, in fact, it is present or, is present in an amount greater than a threshold or designated cut-off concentration. See Appendix 2B.

False Positive Rate: In qualitative analysis, a measure of how often a test result indicates that an analyte is present, when, in fact, it is not present or, is present in an amount less than a threshold or designated cut-off concentration. See Appendix 2B.

Fitness for Purpose: Degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose.

Guidance Level: Level of concern or action level issued under good guidance practices that must be reliably identified or quantified in a sample.

Incurred Samples: Samples that contain the analyte(s) of interest, which were not derived from laboratory fortification but from sources such as exogenous exposure or endogenous origin. Exogenous exposure includes, for example, pesticide use, consumption by an animal, or environmental exposure.

Interference: A positive or negative response or effect on response produced by a substance other than the analyte. Includes spectral, physical, and chemical interferences which result in a less certain or accurate measurement of the analyte.

Intermediate Precision: Within-laboratory precision obtained under variable conditions, e.g., different days, different analysts, and/or different instrumentation.

Internal Standard: A chemical added to the sample, in known quantity, at a specified stage in the analysis to facilitate quantitation of the analyte. Internal standards are used to correct for matrix effects, incomplete spike recoveries, etc. Analyte concentration is deduced from its response relative to that produced by the internal standard. The internal standard should have similar physico-chemical properties to those of the analyte.

Laboratory Fortified Matrix: See *Matrix Spike*.

Level of Concern: Level of concern is the concentration of an analyte in a sample that has to be exceeded before the sample can be considered violative. This concentration can be a regulatory tolerance, safe level, action level, guidance level or a laboratory performance level.

Limit of Detection (LOD): The minimum amount or concentration of analyte that can be reliably distinguished from zero. The term is usually restricted to the response of the detection system and is often referred to as the *Detection Limit*. When applied to the complete analytical method it is often referred to as the *Method Detection Limit* (MDL). Sample calculations are in references [22] and [23].

Limit of Quantitation (LOQ): The minimum amount or concentration of analyte in the test sample that can be quantified with acceptable precision. Limit of quantitation (or quantification) is variously defined but must be a value greater than the MDL and should apply to the complete analytical method. Sample calculations are in references [22] and [23].

Limit Test: A type of semi-quantitative screening method in which analyte(s) has a defined level of concern. Also referred to as binary or pass/fail tests.

Linearity: The ability of a method, within a certain range, to provide an instrumental response or test results proportional to the quantity of analyte to be determined in the test sample.

Matrix: All the constituents of the test sample with the exception of the analyte.

Matrix Blank: A substance that closely matches the samples being analyzed with regard to matrix components. Ideally, the matrix blank does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. The matrix blank is used to determine the absence of significant interference due to matrix, reagents and equipment used in the analysis.

Matrix Effect: An influence of one or more components from the sample matrix on the measurement of the analyte concentration or mass. Matrix effects may be observed as increased or decreased detector responses, compared with those produced by simple solvent solutions of the analyte.

Matrix Source: The origin of a test matrix used in method validation. A sample matrix may have variability due to its source. Different food matrix sources can be defined as different commercial brands, matrices from different suppliers, or in some cases different matrices altogether. For example, if a variety of food matrices with differing physical and chemical properties are selected, the number of sources for each food sample matrix may be one or more.

Matrix spike: An aliquot of a sample prepared by adding a known amount of analyte(s) to a specified amount of matrix. A matrix spike is subjected to the entire analytical procedure to establish if the method is appropriate for the analysis of a specific analyte(s) in a particular matrix. Also referred to as a *Laboratory Fortified Matrix*.

Method blank: A substance that does not contain the analyte(s) of interest but is subjected to all sample processing operations including all reagents used to analyze the test samples. An aliquot of reagent water is often used as a method blank in the absence of a suitable analyte-free matrix blank.

Method Detection Limit (MDL): The minimum amount or concentration of analyte in the test sample that can be reliably distinguished from zero. MDL is dependent on sensitivity, instrumental noise, blank variability, sample matrix variability, and dilution factor.

Method Development: The process of design, optimization and preliminary assessment of the performance characteristics of a method.

Method Validation: The process of demonstrating or confirming that a method is suitable for its intended purpose. Validation criteria include demonstrating performance characteristics such as accuracy, precision, specificity, limit of detection, limit of quantitation, linearity, range, ruggedness and robustness.

Method Verification: The process of demonstrating that a laboratory is capable of replicating a validated method with an acceptable level of performance.

Minimum Detectable Concentration (MDC): In qualitative analysis, an estimate of the minimum concentration of analyte that must be present in a sample to ensure at a specified high probability (typically 95% or greater) that the measured response will exceed the detection threshold, leading one to correctly conclude that an analyte is present in the sample.

Precision: The closeness of agreement between independent test results obtained under specified conditions. The precision is described by statistical methods such as a standard deviation or confidence limit of test results. See also *Random Error*. Precision can be further classified as *Repeatability, Intermediate Precision*, and *Reproducibility*.

Qualitative Analysis/Method: Analysis/method in which substances are identified or classified on the basis of their chemical, biological or physical properties. The test result is either the presence or absence of the analyte(s) in question.

Quantitative Analysis/Method: Analysis/method in which the amount or concentration of an analyte may be determined (or estimated) and expressed as a numerical value in appropriate units with acceptable accuracy and precision.

Random error: Component of measurement error that in replicate measurements varies in an unpredictable manner. See also *Precision*.

Range: The interval of concentration over which the method provides suitable accuracy and precision.

Reagent Blank: Reagents used in the procedure taken through the entire method. Reagent Blanks are used to determine the absence of significant interference due to reagents or equipment used in the analysis.

Recovery: The proportion of analyte (incurred or added) remaining at the point of the final determination from the analytical portion of the sample measured. Usually recovery is expressed as a percentage.

Reference material: A material, sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process or in examination of nominal properties.

Reference standard: A standard, generally having the highest metrological quality available at a given location in a given organization, from which measurements are made or derived. Note: Generally, this refers to recognized national or international traceable

standards provided by a standards producing body such as the National Institute of Standards and Technology (NIST).

Repeatability (RSD_r): Precision obtained under observation conditions at a specific concentration/spike level where independent test results are obtained with the same method on identical test items in the same test facility by the same operator using the same equipment within short intervals of time. Should be included in all quantitative MLV reports.

Representative Analyte: An analyte used to assess probable analytical performance with respect to other analytes having similar physical and/or chemical characteristics. Acceptable data for a representative analyte are assumed to show that performance is satisfactory for the represented analytes. Representative analytes should include those for which the worst performance is expected. Representative analytes are used mostly for non-targeted analysis and unknown screening procedures.

Representative Matrix: Matrix used to assess probable analytical performance with respect to other matrices, or for matrix-matched calibration, in the analysis of broadly similar commodities. For food matrices, similarity is usually based on the amount of water, fats, protein, and carbohydrates. Sample pH and salt content can also have a significant effect on some analytes.

Reproducibility (RSD_R): Precision obtained at a specific concentration/spike level under observation conditions where independent test results are obtained with the same method on identical test items in different test facilities with different operators using different equipment. Should be included in all quantitative MLV reports.

Ruggedness/Robustness: A measure of the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Screening Analysis/Method: An analysis/method intended to detect the presence of analyte in a sample at or above some specified concentration (action or target level). Screening methods typically attempt to use simplified methodology for decreased analysis time and increased sample throughput.

Selectivity: The extent to which a method can determine particular analyte(s) in a mixture(s) or matrix(ces) without interferences from other components of similar behavior. Selectivity is generally preferred in analytical chemistry over the term *Specificity*.

Sensitivity: The change in instrument response which corresponds to a change in the measured quantity (*e.g.*, analyte concentration). Sensitivity is commonly defined as the gradient of the response curve or slope of the calibration curve at a level near the LOQ.

Specificity: In quantitative analysis, specificity is the ability of a method to measure analyte in the presence of components which may be expected to be present. The term *Selectivity* is generally preferred over Specificity.

Spike Recovery: The fraction of analyte remaining at the point of final determination after it is added to a specified amount of matrix and subjected to the entire analytical procedure. Spike Recovery is typically expressed as a percentage. Spike recovery should be calculated for the method as written. For example, if the method prescribes using deuterated internal standards or matrix-matched calibration standards, then the reported analyte recoveries should be calculated according to those procedures.

Standard: A substance of known identity and purity and/or concentration.

Standard Reference Material (SRM): A certified reference material issued by the National Institutes of Standards and Technology (NIST) in the United States. (www.nist.gov/SRM).

Systematic error: Component of measurement error that in replicate measurements remains constant or varies in a predictable manner. This may also be referred to as *Bias*.

Threshold Value: In qualitative analysis, the concentration of the analyte that is either statistically lower than the level of concern (for limit tests) or at which positive identification ceases (for confirmation of identity methods). See also *Cut-off Concentration*.

Trueness: The degree of agreement of the mean value from a series of measurements with the true value or accepted reference value. This is related to systematic error (bias).

Uncertainty: Non-negative parameter characterizing the dispersion of the values being attributed to the measured value.

APPENDIX 2 – Examples of Acceptability Criteria for Certain Performance Characteristics

Examples of acceptability criteria are found in references 7,9,10,14 and 18, Table A below summarizes what is included in references 7 (AOAC) and 10 (CODEX). No single set of acceptability is going to be truly applicable to all methodology covered in the FVM program. For example, a single analyte method, particularly an isotope dilution method, is expected to have better recoveries than a multi-analyte method. However, a good starting point for many methods is found in Table A below and in the Codex Alimentarius Commission, Procedural Manual, Twenty-second ed., 2014 [10]

A. Quantitative Method Acceptability Criteria

Table A2.1. Method Criteria for Method Levels at Increasing Orders of Magnitude (reproduced in part from reference 10, Table 4, p. 72 and reference 7)

ML* unit	0.001 mg/kg	0.01 mg/kg	0.1 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg	1 g/kg	10 g/kg
Alternative ML* unit	1 ppb	10 ppb	100 ppb	1 ppm	10 ppm	100 ppm	0.1%	1 %
Concentration ratio of ML (C_{ML})	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Minimum applicable range	From 0.0006 to 0.0014 mg/kg	From 0.006 to 0.014 mg/kg	From 0.03 to 0.17 mg/kg	From 0.52 to 1.48 mg/kg	From 6.6 to 13.3 mg/kg	From 76 to 124 mg/kg	From 0.83 to 1.2 g/kg	From 8.8 to 11 g/kg
LOD (≤ mg/kg)	0.0002	0.002	0.01	0.1	1	10	100	1000
LOQ (≤ mg/kg)	0.0004	0.004	0.02	0.2	2	20	200	2000
RSD_r**	22%	22%	11%	8%	6%	4%	3%	2%
$PRSD_R$#	22%	22%	22%	16%	11%	8%	6%	4%
RSD_R##	≤ 44%	≤ 44%	≤ 44%	≤ 32%	≤ 22%	≤ 16%	≤ 12%	≤ 8%
Recovery	40%-120%	60%-115%	80%-110%	80%-110%	80% - 110%	90% - 107%	95% – 105%	97%-103%

* ML is a method level and can be defined for the analyte(s)/sample matrix(ces) combination as a maximum level, minimum level, normative level or concentration range depending on the intended use of the method.

**The RSD_r or Repeatability Precision refers to the degree of agreement of results when conditions are maintained as constant as possible within a short period of time (e.g., relative standard deviation of replicates or best precision exhibited by a single laboratory). Typically, acceptable values for RSD_r are between ½ and 2 times the value shown (Horwitz Ratio ($HorRat_r$) = RSD_r (found, %)/ RSD_r (calculated, %)). For concentration ratios ≥ 10^{-7} Horwitz theory is applied [10]. For concentration ratios < 10^{-7}, Thompson theory is applied [10].
#The $PRSD_R$ or Predicted Relative Reproducibility Standard Deviation is based on the Horwitz/Thompson equation. For concentration ratios < 10^{-7}, Thompson theory is applied [10].
The RSD_R or Reproducibility Precision refers to the degree of agreement of results when operating conditions are as different as possible (*e.g.*, same test samples in different laboratories) and should be calculated from the Horwitz/Thompson equation. When the Horwitz/Thompson equation is not applicable (for an analytical purpose or according to a regulation) or when "converting" methods into criteria then it should be based on the RSD_R from an appropriate method performance study. The ratio between the found and predicted value should be ≤ 2. ($HorRat_R = RSD_R / PRSD_R \leq 2$)

B. Qualitative Method Acceptability Criteria

Example statistical approach to confirm false negative (FN) and false positive (FP) rates as <5%

Zero acceptance number sampling is a statistical approach commonly used to test a hypothesis (or criteria) for the frequency of defective items in a population (e.g., such as FN or FP rates with repeated testing). For this approach, all tested samples must have the correct response in order to accept the hypothesis (i.e., accept only when zero "defective" responses observed). The minimum number of samples that must be tested depends on the criteria for the defect rate and the level of statistical confidence:

$$n = \log(\alpha) / \log(1-p)$$

where 1-α is the confidence level and *p* is the maximum acceptable defect rate per sample (e.g., FN or FP rate). Sample sizes to assess selected criteria for FN or FP rates with varying levels of confidence are provided in the following table.

Table A2.3. **Samples required to determine false positive/negative rates**

	Confidence Level			
FN or FP rate	80%	90%	95%	99%
<1%	161	230	299	459
<2%	80	114	149	228
<5%	***32***	***45***	***59***	***90***
<10%	16	22	29	44

For example, if the goal is to have 95% confidence that the FN rate is <5% then test 59 samples with the analyte present at the concentration of interest, typically the LOD or a relevant level of concern, in a range

of matrices. The criteria are satisfied if all 59 test results are positive for the target.

This sample size formula is related to the Clopper-Pearson confidence interval for Binomial proportions and frequently used for zero defect acceptance sampling plans for commodity lots. The rationale for the sample size is that when the probability of a defective (incorrect) test response is p for each sample then $(1 - p)^n$ is the probability that n samples will have the correct response. The minimum sample size required for a specified level of confidence follows from setting the probability of that outcome equal to the type I error rate α and solving for n.

APPENDIX 3 - Examples of Validation Plans

A. Extension to other matrices with the same analyte(s) at Level One Validation

This scheme represents an emergency use method extension plan for Matrix Y and Analyte Z. This plan utilizes two different sources of matrix. *In cases where a representative matrix is being used to characterize a whole family of commodities, it is recommended that additional, different commodities from that family are used as "sources".* Note that this plan is for emergency use only – the new matrix (or matrices) cannot be officially included in the scope of the method until at the minimum a Level Two Validation is performed.

Table A3.1. Plan for Matrix Extension (Level One Validation, Example)

	Matrix	Samples 1 & 2	Analyte Z Fortified Samples 3 & 4	Analyte Z Fortified Samples 5 & 6	Analyte Z Fortified Samples 7 & 8
Day 1	Matrix Y (Source 1)	Blank	½X Spike Level	X Spike Level	2X Spike Level
Day 1	Matrix Y (Source 2)	Blank	½X Spike Level	X Spike Level	2X Spike Level

Notes:
i. Test portion matrices listed as Matrix Y represent 2 different commercial brands.
ii. Fortification levels: fortification will be at the level of concern or action level (X) as stated in the method and at levels corresponding to 1/2X and 2X.
iii. Fortification of each matrix can be done on the same day.
iv. Other fortification plans meeting requirements specified in Table 1 may be used.

B. Extension to similar analytes in the same matrix at Level Two Validation

A validated method can be extended to other potential analyte(s) belonging to the same chemical group. For example, a toxin method can be extended to other toxins. An example of the composition of a set of validation studies for method extension is shown in the following table for new analytes Y and Z in canned corn from 3 different sources where the method is validated originally for analyte A in corn.

Table A3.2. Plan for Extension to Similar Analytes (Level Two Validation, Example)

	Matrix	Analyte Y fortification levels	Analyte Z fortification levels
Day 1	Corn 1,2,3	0, 1/2X, X, 2X	0, 1/2X, X, 2X
Day 2	Corn 1,2,3	0, 1/2X, X, 2X	0, 1/2X, X, 2X
Day 3	Corn 1,2,3	0, 1/2X, X, 2X	0, 1/2X, X, 2X

Notes:
i. Three different commercial brands of same product will be analyzed.
ii. Fortification levels: fortification will be at the level of concern or action level (X) as stated in the method and at levels corresponding to 1/2X and 2X.
iii. Each analyte will be analyzed in blank matrix and in duplicate at 1/2X, X and 2X fortification levels.
iv. Simultaneous analysis of the analytes can be undertaken if warranted.
v. Other fortification plans meeting requirements specified in Table 1 may be used.

C. Validation at Level Two for single matrix and single analyte

This plan utilizes 3 different commercial brands of one matrix. The single matrix is being validated for a single analyte.

Table A3.3. Plan for Single Matrix and Single Analyte Level Two Validation (Example)

	Matrix 1 Source 1	Matrix 1 Source 2	Matrix 1 Source 3
Day 1	Blank Fortified (X)	Fortified (X) Fortified (2X)	Blank Fortified (1/2X)
Day 2	Fortified (2X) Fortified (1/2X)	Blank Fortified (1/2X)	Blank Fortified (2X)
Day 3	Fortified (1/2X) Fortified (X)	Fortified (2X) Blank	Fortified (X) Fortified (X)
Day 4	Fortified (2X) Blank	Fortified (X) Fortified (1/2X)	Fortified (2X) Fortified (1/2X)

Notes:
i Sample matrix, represents one matrix from 3 different sources of matrix.
ii Fortification levels: fortification will be at the level of concern or action level (X) as stated in the method and at levels corresponding to 1/2X and 2X.
iii Each of 3 different sources of matrix will be analyzed 8 times (replicate analyses) over the course of experiment, two times unfortified, two times fortified at each level.
iv. The validation in this example will take place over a period of 4 days. It is acceptable to complete the validation in a single day.
v. Other fortification plans meeting requirements specified in Table 1 may be used.

APPENDIX 4 – Selection of Representative Matrices

Two tools that can aid in selection of representative matrices and CRMs when designing a validation protocol for a method intended to have applicability to a broad scope of products are shown below. Food composition varies greatly, and the performance of some methods is more impacted than others by differences in matrix composition, making the validation of methods intended for a wide variety of foods a difficult balance between available resources and sufficient validation with a variety of food types.

A. Commodity groups and representative commodities

Table A4.1. Vegetable and Fruits, Cereals and Food of Animal Origin (reproduced in part from reference 14)

Commodity groups	Typical commodity categories	Typical representative commodities
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches
	Other fruit	Bananas
	Alliums	Onions, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, Brussels sprouts, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Fresh legume vegetables	Fresh peas with pods, peas, mange tout, broad beans, runner beans, French beans
	Fresh Fungi	Champignons, canterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrots, potatoes, sweet potatoes
2. High acid content and high water content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, black currant, red currant, white currant, grapes

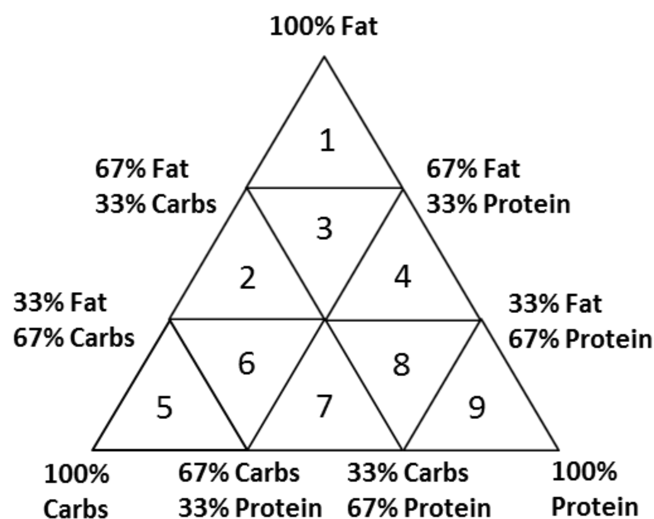
Table A4.1. Vegetable and Fruits, Cereals and Food of Animal Origin (continued)

Commodity groups	Typical commodity categories	Typical representative commodities
3. High sugar and low water content	Honey, dried fruit	Honey, raisins, dried apricots, dried plums, fruit jams
4a. High oil content and very low water content	Tree nuts	Walnuts, hazelnuts, chestnuts
	Oil seeds	Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame, etc.
	Pastes of tree nuts and oil seeds	Peanut butter, tahini, hazelnut paste
4b. High oil content and intermediate water content	Oily fruits and products	Olives, avocados and pastes thereof
5. High starch and/or protein content and low water and fat content	Dry legume vegetables/pulses	Field beans, dried broad beans, dried haricot beans (yellow, white/navy, brown, speckled), lentils
	Cereal grain and products thereof	Wheat, rye, barley and oat grain; maize, rice, whole meal bread, white bread, crackers, breakfast cereals, pasta, flour.
6. “Difficult or unique commodities”		Hops, cocoa beans and products thereof, Coffee, tea, spices
7. Meat (muscle) and Seafood	Red muscle	Beef, pork, lamb, game, horse
	White muscle	Chicken, duck, turkey
	Offal	Liver, kidney
	Fish	Cod, haddock, salmon, trout
8. Milk and milk products	Milk	Cow, goat and buffalo milk
	Cheese	Cow and goat cheese
	Dairy products	Yogurt, cream
9. Eggs	Eggs	Chicken, duck, quail, and goose eggs
10. Fat from food of animal origin	Fat from meat	Kidney fat, lard
	Milk fat	Butter

B. AOAC Food Matrix Triangle

The AOAC Food Matrix Triangle (Figure A4.1) can be used to categorize foods and food matrix reference materials into nine sectors based on relative fat, protein and carbohydrate content [9, 19, 20]. This tool can be useful in the validation of methods intended for a wide variety of food matrices and to help in categorizing similar food matrices for methods intended for more limited applicability.

Figure A4.1. Foods Partitioned into Sectors Based on Their Protein, Fat, and Carbohydrate Content



APPENDIX 5 – Verifications and Extensions of Existing Methods

Method Verification [24]: Method verification is a demonstration that a laboratory can properly perform a standard method that has been previously validated elsewhere. Verification of a quantitative or qualitative method that has undergone Multi-Laboratory Validation (Level III or Level IV) through the established food and feed program process, as well as compendial methods that have undergone MLVs that meet or exceed the requirements set in the Chemical Methods Validation Guidelines, requires analysis of spikes at two concentration levels, each extracted and run in triplicate, along with a matrix blank (when available) and a method blank. A single matrix can be selected even if the original method is applicable to multiple matrices. The selected spiking concentrations (reference materials should be used, if available) should ensure that the method meets the requirements of the particular Program Area and consider any relevant regulatory limits/action levels (e.g. spiking at 0.5x any applicable limit). These spikes should be run prior to the analysis of regulatory samples. For some analytes, spiking with pure standard alone does not sufficiently demonstrate method performance (e.g., BPA in can coatings contain oligomeric interferences; gluten in fermented/hydrolyzed products; protein-bound veterinary drug metabolites do not perform the same as unbound analytes, elemental analysis of matrices resistant to digestion). In these cases, reference materials and/or real samples should be analyzed, in place of spikes, to demonstrate method performance. Method performance results should be

approved by the supervisor, local QA manager (if applicable), and Laboratory Director, and shared with the ORA/ORS Program Coordinator. Results should also be shared with the MLV organizer/ORA/Center Subject Matter Expert (SME) (when possible), and the appropriate TAG for review if/when additional evaluation is needed.

Verification of a quantitative or qualitative method that has NOT undergone Multi-Laboratory Validation (Level III or Level IV) through the established food and feed program process requires Level II SLV unless the method is intended for one time or emergency use, in which case analyzing two matrix spike levels (each in triplicate) along with a matrix blank and a method blank is acceptable. Verifications should be performed prior to the analysis of regulatory samples. Spiking requirements are summarized in Table 1.

Validation Level of the Original Method (Quantitative or Qualitative)	Minimum Requirements for Verification	Notes
Level II SLV: to be used routinely/long term by adopting laboratory	Level II SLV	Must be run prior to the analysis of regulatory samples
Level II SLV: for one time/short term/emergency use by adopting laboratory	Two matrix spike levels, run in triplicate, along with a matrix blank and a method blank	Must be run prior to the analysis of regulatory samples
Level III MLV or Level IV Collaborative Study or equivalent compendial method	Two matrix spike levels, run in triplicate, along with a matrix blank and a method blank	Must be run prior to the analysis of regulatory samples

Table 1: Guidance for Method Verifications

Matrix Extensions: It is critical to note that it is impossible to provide exhaustive guidance on when a matrix extension is required. For example, Elemental Analysis Manual Method 4.10 was validated for grape, pear, and apple juice, but required modification to perform acceptably for pomegranate, cherry, and prune juice. The perspective of analysts with subject matter expertise and close monitoring of QA/QC data are necessary to ensure differences in method performance in different matrices are identified, and that the method is fit for use.

The identification and classification of a new matrix is dependent on the programmatic area. Pesticides should refer to Appendix 4, Table 1 of the Guidelines for the Validation of Chemical Methods for the FDA FVM Program. For DNA identification methods, consult the Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, and Cosmetics. Other program areas should contact the ORA/ORS

Program Coordinator, the ORA/Center SME, and the ORA/ORS Office of Research Coordination and Evaluation (ORCE) for guidance. All spiking concentrations used should ensure that the method meets the requirements of the particular Program Area and consider any relevant regulatory limits/action levels. The results should be shared with the local QA manager and the ORA/ORS Program Coordinator.

If the original method uses isotopically labeled internal standards for each analyte of interest or matrix matched calibration curves, a spike (in duplicate) along with a matrix blank analyzed concurrently with a regulatory sample is sufficient to demonstrate suitable performance (see Table 2). For other methods, spikes at two concentrations, each analyzed in duplicate, along with a matrix blank should be performed. This can be performed concurrently with or prior to the analysis of regulatory samples. All recoveries should be within the range of those reported for the matrices in the original validation, and consistent with Appendix 2A of the Guidelines for the Validation of Chemical Methods for the FDA FVM Program. The results should be shared with the lab supervisor, the local QA manager, and the ORA/ORS program coordinator. Once completed, the matrix can now be analyzed by other regulatory labs using the same harmonized method without further validation.

Technique used in the Original Method	Minimum Requirements for Matrix Extensions	Notes
Methods using isotopically labeled internal standards or matrix matched calibration curves	Spike run in duplicate, along with a matrix blank (if available).	Can be run prior to or concurrent with regulatory samples
All other methods	Two matrix spike levels, run in duplicate, along with a matrix blank (if available)	Can be run prior to or concurrent with regulatory samples

Table 2: Guidance for Matrix Extensions

Analyte Extensions: For the addition of a new analyte (quantitative or qualitative) to an existing validated method, a Level II SLV should be undertaken for that analyte, as well as determinations of LOD, LOQ, and linearity. In cases where the method performance for existing analytes may be impacted (e.g. optical methods, analytes with similar chromatographic retention, isobaric target analytes in mass spectrometry, duty cycle issues with mass spectrometry, multiplexed antibody-based methods), the validation must ensure standards continue to perform acceptably for those existing analytes. This must include confirming the absence of interferences and maintenance of linearity and sensitivity for all existing analytes. In the case of mass spectrometric methods, a sufficient number of data points (>10) must be maintained for quantitation of all analytes. All recoveries should be

consistent with Appendix 2A of the Guidelines for the Validation of Chemical Methods for the FDA FVM Program. The results should be shared with the lab supervisor, the local QA manager, and the ORA/ORS Program Coordinator. Assuming methods are harmonized between multiple laboratories, this will only need to be performed in one laboratory. Once completed, the analyte can now be analyzed by other regulatory labs using the same harmonized method following a verification.

Platform Extensions: When switching to a new platform that applies the same technique as used in the initially validated quantitative or qualitative method (e.g. an Agilent LC-QQQ to an AB SCIEX LC-QQQ), provided the remainder of the original method is unchanged, a full validation is unnecessary. However, analysts should evaluate the LOD/LOQ to ensure they are still suitable and determine that the original calibration curve fit and linear dynamic range have not changed (e.g., the curve has not become a quadratic/reached saturation). The results should be shared with the lab supervisor, the local QA manager, and the ORA/ORS Program Coordinator.

When switching a method to a new instrument (e.g., LC-MS/MS to LC-Q Exactive, ICP-Q to ICP-QQQ, GC-MS to GC-MS/MS), a Level II SLV should be performed for all target analytes (performed by one lab, verified by additional labs). This can be performed via the determination of spike recoveries, or by standard addition to extracted matrices, provided the number of samples meets or exceeds the requirements of a Level II SLV. Analysts should also evaluate the LOD/LOQ and linear dynamic range for all analytes to ensure they still meet the needs of the particular Program. The results should be shared with the lab supervisor, the local QA manager, and the ORA/ORS Program Coordinator. Once completed, the new platform can now be used by other regulatory labs using the same harmonized method following a verification.

APPENDIX 6 – Acceptable Modifications to Mass Spectrometry Methods

Modification Guidelines for Chromatography - Mass Spectrometry Methods

1.0: Scope

This document establishes guidance on the acceptable instrumental modifications to liquid chromatography- and gas chromatography-mass spectrometry methods for determination of chemical analytes in food, feed and cosmetics.

2.0 Introduction/Applicability

Multi-laboratory validation (MLV) studies are performed to ensure that the methodology will accurately measure, within an acceptable precision, the target analyte(s) in matrices defined in the method scope. Subsequent use of a validated method for regulatory testing, requires that the analyst perform the analysis, (sample preparation and instrumental analysis) as described in the validated method. Therefore, when utilizing a validated method, modification to the method procedure should not be made except in cases of critical necessity. The guidelines presented herein address changes to the analytical conditions (e.g., chromatographic separation and/or mass spectrometric detection) and method parameters that are acceptable within the boundaries

of a validated method. In situations where a change to the validated method falls outside the scope of the original method or the ranges described herein, additional testing is required. Depending on the change, these additional tests may either extend the original scope of the method or require the identification of an entirely new method.

These guidelines are applicable to methods being performed on the same analytes, matrices/matrix type and the same mass spectrometer (make and model) as described in the MLV. These guidelines **do not** address extension of methods to new instrument platforms (i.e., different make or model), extension to new analytes, extension to new matrices/matrix types or changes to the sample preparation procedures.

If a validated method specifically addresses any of the modifications listed in these guidelines, then the statements in the method supersede these general guidelines.

2.1 Applicability to Collaborative Studies

Participation in a multi-laboratory collaborative study involves strict adherence to the developed method procedure. Modifications from the collaborative study protocol should be avoided except in cases of critical necessity. When a modification is required, even if within the limits listed in these guidelines, it should be reviewed and agreed to by the study director and must be documented in the final validation report for review by the CMVS. If a modification falls outside the limits listed in these guidelines, additional testing (e.g., additional lab) or the removal of the data from the collaborative study may be required based on the review and recommendations made by the CMVS and/or CRCG.

During the running of an MLV, deviations (i.e., unplanned modifications) can occur. These guidelines can be used by the study director and the CMVS to determine the impact of each deviation. If the deviation falls within the limits listed in these guidelines, additional testing should not be required. However, the deviation must still be captured in the final validation report. If the deviation falls outside of the limits listed in these guidelines, additional testing (e.g., additional lab) or the removal of the data from the collaborative study may be required based on the review and recommendations made by the CMVS and/or the CRCG.

3.0 Liquid Chromatography-Mass Spectrometry Modifications

3.1 Acceptable Modifications

The following modifications represent minor changes to a method that may be made if critically required. All modifications should be shared with the local QA manager and reported through the appropriate TAG. The modifications listed below do not need to be captured as part of the scope of the compendial method.

A. **Liquid Chromatography Column**: The specific column(s) (manufacturer, bonded phase, particle size, particle type, pore size) identified in the multi-laboratory validated method should be used when running the method. The column dimensions (either length or diameter, not both) can be altered if subsequent changes are made to the flow rate to achieve the same separation reported in the validated method (relative retention time and chromatographic resolution ±20%). If comparable chromatography cannot be achieved

with the new column/flow rate, further testing is required before the column can be used (Section 3.2A).

B. Mobile Phase Modifiers: Small changes in the concentration of mobile phase modifiers (e.g., salts) (±10 %) and pH (± 0.2 units), except ion pair reagents, are acceptable and should be within the robustness of a well-developed method. This applies to both gradient and isocratic separations. Chromatographic separation should be maintained (relative retention time and chromatographic resolution ±20 %).

C. Injection Volume: Given possible differences in the performance characteristics between instruments or in response to changes made to the column dimensions and flow rates (Section 3.1A), it may be necessary to change the sample injection volume. The change should not impact peak symmetry, resolution, and method sensitivity (no statistical difference at 95% CL). The analyst must verify that the calibration range is maintained (no statistically significant variation) or improved.

D. Reproduction of HPLC methods UHPLC instrumentation: Methods developed and validated with HPLC hardware may be reproduced using modern UHPLC equipment, as long as the original column is utilized in the UHPLC system.

E. Source Conditions: Instruments of the same series from the same vendor (i.e., Sciex 6500 QTRAP) may have performance differences which would require different source conditions (e.g., temperature, gas flow rate) to be used to meet the same performance specifications. Generally, these differences are small and therefore any changes to the source conditions should be minor adjustments to temperature, voltages and/or gas flows. Additionally, analysts should avoid making multiple changes that could have detrimental additive effects, such as reducing both temperature and gas flow. The new source conditions should maintain (or improve) the calibration range. If any loss (statistically significant) in the calibration range is detected then matrix extracts ($n \geq 2$ replicates per validated matrix) at critical concentrations (e.g., Level of Concern, LOQ) should be evaluated to establish that under the new conditions the analytes can be accurately quantitated at these critical concentrations. Any changes to the conditions should be recorded for use by other analysts on the system.

F. Collision Cell: A change to the collision gas and/or the collision energy is/are allowable and may be necessary. The impact of the new collision cell conditions should be evaluated on calibration standards across the entire calibration range. The new collision cell conditions should maintain (or improve) the calibration range. If any loss (statistically significant) in the calibration range is detected then matrix extracts ($n \geq 2$ replicates per validated matrix) at critical concentrations (e.g., Level of Concern, LOQ) should be evaluated to establish that under the new conditions the analytes can be accurately quantitated at these critical concentrations. Any changes to the conditions should be recorded for use by other analysts on the system.

G. Mass Spectra Ion Monitoring Window: The time and width of ion monitoring window may be adjusted to account for changes in the chromatographic elution profile. The number of concurrent transitions should not be changed.

H. **Number of Analytes Monitored**: The number of analytes monitored may be reduced during a confirmatory run, check analysis, or to perform the analysis on a smaller number of target analytes. To increase the number of analytes an Analyte Extension Study as described in *Appendix 5 Verifications and Extensions of Existing Methods* of the *Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Ed.* should be performed.

3.2 Liquid Chromatography-Mass Spectrometry Modifications that Require Specific Extension Study or Verification Study

A. **Liquid Chromatography Column**: The column used in the original MLV should be used when performing the method. If a critical need arises, such as discontinuation of the analytical column used in the validation, or unavailability due to supply issues, it may be necessary to use a different column. The new column must use the same bonded phase chemistry (e.g., C18), particle type (e.g., solid-core or porous), similar particle and pore size (± 30%) and provide the same elution order. The separation performance of the potential column should be compared to the reference separation reported in the validated method, choosing the column that best reproduces the reference separation. The method modification and associated verification or validation data, as described below, must be collected, reviewed by the CRCG and added as an addendum to the MLV or documented in the method compendium as an extension to the scope of the method. If a new column produces a different separation (i.e., elution order) or does not meet these requirements, then it is deemed a different method and the column cannot be added as an addendum to the original method. The studies below also apply to columns of the same characteristics but different dimensions, where the separation of the original method cannot be duplicated (Section 3.1A).

1. Single Analyte Method or Multi-analyte method without stable isotope or non-coeluting* stable isotope internal standard: A method verification study as described in *Appendix 5 Verifications and Extensions of Existing Methods* of the *Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Ed.* should be performed on the new column.

2. Single Analyte Method or Multi-analyte method Multi-analyte with coeluting stable isotope internal standard: A platform extension (new platform) study as described *Appendix 5 Verifications and Extensions of Existing Methods* of the *Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Ed.* should be performed on the new column.

   * This is not intended to be a comparison to the original method, therefore even if the stable isotope internal standard does not coelute in the original method the studies described should be performed.

3.3 Liquid Chromatography-Mass Spectrometry Modifications that Require Further Validation (i.e., create a new method)

The following characteristics of the method cannot be changed. Any change would represent a new method, which should undergo the appropriate validation according to Section 2.5 of the Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd Ed. prior to

use.

a. Column Characteristics (e.g., separation mode [reverse phase to normal phase], particle type)
b. Column Setpoint Temperature
c. Data Collection Mode (e.g., Full Scan, MRM, DDA): There may be changes to data collection modes that would not constitute a new method. Requests to allow a change should be submitted for review by the CRCG.
d. Ion Pair Reagent Composition
e. Ionization Polarity
f. Ion Selection of precursor and product ions: For confirmatory analysis the use of additional structurally significant products ions is allowable, provided they are compared to a standard analyzed at the time of use and do not reduce the dwell times of the quantifying and qualifying ions listed in the method.
g. Ionization Source (e.g., ESI, APCI)
h. Mass Resolution
i. Mobile Phase (composition and gradient, except for changes related to Section 3.1A)

4.0 Gas Chromatography-Mass Spectrometry Modifications

4.1 Acceptable Modifications

The following modifications represent minor changes to a method that may be made if critically required. All modifications should be shared with the local QA manager and reported through the appropriate TAG. The modifications listed below do not need to be captured as part of the scope of the compendial method.

A. Gas Chromatography Column: The specific column used in the initial method development and validation is the preferred column option. Columns of the same dimensions and stationary phase (e.g., chemistry, thickness) but from different vendors can be used in place of the original column. The separation characteristics should be evaluated to ensure they remain consistent with the original method (relative retention time and chromatographic resolution ±20%). When necessary, it is acceptable to shorten the column during routine maintenance to maintain chromatographic performance. Such changes and the means for assessing chromatographic performance should be captured as part of routine laboratory QA.

B. Injection Volume: Given possible differences in performance characteristics between instruments, it may be necessary to change the injection volume. Any increase should not exceed 2x the validated injection volume and any decrease should not exceed 0.5x the validated injection volume. For split injections, a change to split ratio is permitted but should not exceed 30%. The change should not impact peak symmetry, resolution, and method sensitivity (no statistical difference 95% CL). The analyst must verify that the calibration range is maintained (no statistically significant variation) or improved.

C. Collision Cell: A change to the collision gas and/or the collision energy is/are allowable and may be necessary. The impact of the new collision cell conditions should be evaluated on calibration standards across the entire calibration range. The new collision cell conditions should maintain (or improve) the calibration range. If any loss (statistically significant) in the calibration range is detected then matrix extracts (n≥2 per validated

matrix) at critical concentrations (e.g., Level of Concern, LOQ) should be evaluated to establish that the new conditions can accurately determine the analytes at the relevant concentrations.

D. **Inlet Pressure or Flow Rate or Linear Velocity:** When using a new column, the inlet pressure, flow rate and linear velocity should be set to the values defined in the collaboratively studied method. However, with use, and to maintain method performance, many GC methods may require shortening of the column during routine maintenance. After column maintenance, changes in inlet pressure or flow rate or linear velocity may be required to maintain chromatographic performance. Such changes and the means for assessing chromatographic performance should be captured as part of routine laboratory QA.

4.3 Gas Chromatography-Mass Spectrometry Modifications that Require Further Validation (i.e., create a new method)

The following characteristics of the method cannot be changed. Any change would represent a new method, which should undergo appropriate validation according to Section 2.5 of the Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd Ed. prior to use.

a. Carrier Gas
b. Column Stationary Phase Chemistry (e.g., cyanopropyl to phenyl)
c. Data Collection Mode (e.g., Full Scan, MRM, DDA): There may be changes to data collection modes that would not constitute a new method. Requests to allow a change should be submitted for review by the CRCG.
d. Injection Type
e. Inlet Pressure or Flow Rate or Linear Velocity with new column (See Section 4.2D)
f. Ionization Mode (e.g., EI, CI) CI reagent and purity
g. Ionization Polarity
h. Ion Selection of precursor and product ions (including isolation width): For confirmatory analysis the use of additional structurally significant products ions is allowable, provided they are compared to a standard analyzed at the time of use and do not reduce the dwell times of the quantifying and qualifying ions listed in the method.
i. Mass Spectrometer Resolution
j. Mass Spectrometer Source Conditions
k. Temperature Program

5.0 Further Guidance

It is critical to note that it is impossible to provide comprehensive guidance across all methods, which will ensure that a modification to a method will yield comparable results. Therefore, if the modifications allowed in this document lead to changes in method performance, they should not be implemented as an addendum to the original method and should be communicated to the TAG, and the ORA/ORS Program Coordinator. The perspective of analysts with subject matter expertise, and close monitoring of QA/QC data, is necessary to ensure differences in method performance are adequately assessed, and that the method is fit for use.

All verification or validation results should be shared with the local QA manager and the

ORA/ORS Program Coordinator. Any SLV, that may constitute a method extension must be submitted to the CRCG for review and consideration as an addendum to the MLV. Any changes initiated due to critical need (e.g., discontinuation of a column) should be reported to QA manager, ORA/ORS program coordinator and the CRCG.

6.0 Acronyms

APCI	Atmospheric Pressure Chemical Ionization
CI	Chemical Ionization
CL	Confidence Level
CMVS	Chemical Methods Validation Subcommittee
CRCG	Chemical Research Coordination Group
DDA	Data Dependent Acquisition
EI	Electron Ionization
ESI	Electrospray Ionization
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
LC	Liquid Chromatography
LOQ	Limit of Quantitation
MLV	Multi-Laboratory Validation
MRM	Multiple Reaction Monitoring
ORA/ORS	Office of Regulatory Affairs/Office of Regulatory Science
QA	Quality Assurance
SLV	Single Laboratory Validation
TAG	Technical Advisory Group
UHPLC	Ultra-High Performance Liquid Chromatography

Appendix D: Guidelines for Collaborative Study Procedures To Validate Characteristics of a Method of Analysis

{*Note*: These guidelines incorporate symbols, terminology, and recommendations accepted by consensus by the participants at the IUPAC Workshop on Harmonization of Collaborative Analytical Studies, Geneva, Switzerland, May 4–5, 1987 [*Pure Appl. Chem.* **60**, 855–864(1988); published as "Guidelines for Collaborative Study of Procedure to Validate Characteristics of a Method of Analysis," *J. Assoc. Off. Anal. Chem.* **72**, 694–704(1989)]. The original guidelines were revised at Lisbon, Portugal, August 4, 1993, and at Delft, The Netherlands, May 9, 1994, *Pure Appl. Chem.* **67**, 331–343(1995). These revised, harmonized guidelines have been adopted by AOAC INTERNATIONAL as the guidelines for the AOAC Official Methods Program, *J. AOAC Int.* **78**(5), 143A–160A(1995). Although the directions were developed for chemical studies, some parts may be applicable to all types of collaborative studies.}

Summary Statement of AOAC Recommendation for Design of a Collaborative Study

Minimum Criteria for Quantitative Study

Minimum number of materials (see Note 1 on p. 4).—Five (only when a single level specification is involved for a single matrix may this minimum be reduced to 3).

Minimum number of laboratories.—Eight reporting valid data for each material (only in special cases involving very expensive equipment or specialized laboratories may the study be conducted with a minimum of 5 laboratories, with the resulting expansion in the confidence interval for the statistical estimates of the method characteristics).

Minimum number of replicates.—One, if within-laboratory repeatability parameters are not desired; 2, if these parameters are required. Replication should ordinarily be attained by blind replicates or split levels (Youden pairs).

Minimum Criteria for Qualitative Analyses

Ten laboratories reporting on 2 analyte levels per matrix, 6 test samples per level, and 6 negative controls per matrix. (*Note*: AOAC criteria for qualitative analyses are not part of the harmonized guidelines.)

1. Preliminary Work (Within One Laboratory)

1.1 Determine Purpose and Scope of the Study and Method

Determine purpose of the study (e.g., to determine attributes of a method, proficiency of analysts, reference values of a material, or to compare methods), the type of method (empirical, screening, practical, reference, definitive), and the probable use of the method (enforcement, surveillance, monitoring, acceptance testing, quality control, research). Also, on the basis of the relative importance of the various method attributes (bias, precision, specificity, limit of determination), select the design of the collaborative study. The directions in this document pertain primarily to determining the precision characteristics of a method, although many sections are also appropriate for other types of studies.

Alternatives for Method Selection

(*1*) Sometimes obvious (only method available).

(*2*) Critical literature review (reported within-laboratory attributes are often optimistic).

(*3*) Survey of laboratories to obtain candidate methods; comparison of within-laboratory attributes of candidate methods (sometimes choice may still not be objective).

(*4*) Selection by expert [AOAC-preferred procedure (selection by Study Director with concurrence of General Referee)].

(*5*) Selection by Committee (ISO-preferred procedure; often time-consuming).

(*6*) Development of new method or modification of existing method when an appropriate method is not available. (Proceed as a research project.) (This alternative is time-consuming and resource-intensive; use only as a last resort.)

1.2 Optimize Either New or Available Method

Practical Principles

(*1*) Do not conduct collaborative study with an unoptimized method. An unsuccessful study wastes a tremendous amount of collaborators' time and creates ill will. This applies especially to methods that are formulated by committees and have not been tried in practice.

(*2*) Conduct as much experimentation within a single laboratory as possible with respect to optimization, ruggedness, and interferences. Analysis of the same material on different days provides considerable information on variability that may be expected in practice.

Alternative Approaches to Optimization

(*1*) Conduct trials by changing one variable at a time.

(*2*) Conduct formal ruggedness testing for identification and control of critical variables. *See* Youden and Steiner (pp 33–36, 50–55). The actual procedure is even simpler than it appears. (This is an extremely efficient way for optimizing a method.)

(*3*) Use Deming simplex optimization to identify critical steps. *See* Dols and Armbrecht. The simplex concept can be used in the optimization of instrument performance and in application to analytical chemical method development.

1.3 Develop Within-Laboratory Attributes of Optimized Method

(Some items can be omitted; others can be combined depending on whether study is qualitative or quantitative.)

Determine calibration function (response vs concentration in pure or defined solvent) to determine useful measurement range of method. For some techniques, e.g., immunoassay, linearity is not a prerequisite. Indicate any mathematical transformations needed.

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Determine analytical function (response vs concentration in matrix, including blank) to determine applicability to commodity(ies) of interest.

Test for interferences (specificity): (*1*) Test effects of impurities, ubiquitous contaminants, flavors, additives, and other components expected to be present and at usual concentrations. (*2*) Test nonspecific effects of matrices. (*3*) Test effects of transformation products, if method is to indicate stability, and metabolic products, if tissue residues are involved.

Conduct bias (systematic error) testing by measuring recoveries of analyte added to matrices of interest and to extracts, digests, or other treated solutions thereof. (Not necessary when method defines property or component.)

Develop performance specifications for instruments and suitability tests for systems (which utilize columns or adsorbents) to ensure satisfactory performance of critical steps (columns, instruments, etc.) in method.

Conduct precision testing at the concentration levels of interest, including variation in experimental conditions expected in routine analysis (ruggedness). In addition to estimating the "classical" repeatability standard deviation, s_r, the initiating laboratory may estimate the total within-laboratory standard deviation (s_e) whereby s_e is the variability at different days and with different calibration curves, by the same or different analysts within a single laboratory. This total within-laboratory estimate reflects both between-run (between-batch) and within-run (within-batch) variability.

Delineate the range of applicability to the matrices or commodities of interest.

Compare the results of the application of the method with existing, studied methods intended for the same purposes, if other methods are available.

If any of the preliminary estimates of the relevant performance of these characteristics are unacceptable, revise the method to improve them, and re-study as necessary.

Have method tried by analysts not involved in its development.

Revise method to handle questions raised and problems encountered.

1.4 Prepare Description of Method

Note: A collaborative study of a method involves practical testing of the written version of the method, in its specific style and format, by a number of laboratories on identical materials.

Prepare method description as closely as possible to format and style that will be used for eventual publication.

Clearly specify requirements for chromatographic materials, enzymes, antibodies, and other performance-related reagents.

Clearly describe and explain every step in the analytical method so as to discourage deviations. Use imperative directions; avoid subjunctive and conditional expressions as options as far as possible.

Clearly describe any safety precautions needed.

Edit method for completeness, credibility (e.g., buffer pH consistent with specified chemicals, volumes not greater than capacity of container), continuity, and clarity.

Check for inclusion of performance specifications and system suitability tests, defined critical points, and convenient stopping points. Incorporate physical or chemical constants of working standards solutions, e.g., absorptivities, half-scale deflections, recoveries, etc., or properties of operating solutions and chromatographic materials, e.g., pH, volumes, resolution, etc., and any other indicators (e.g., sum equals 100%) that suggest analysis is proceeding properly.

If time and resources are available, conduct pilot study involving 3 laboratories.

1.5 Invite Participation

Selection of Collaborators/Candidate Laboratories

Laboratories invited to participate should have personnel experienced in the basic techniques employed; experience with the method itself is not a prerequisite for selection. Lists of possible participants can be developed through personal contacts, technical societies, trade associations, or literature search, and advertisements in the Referee section of AOAC's magazine, *Inside Laboratory Management*. Collaborators are chosen by the organizers of the collaborative study from a diversity of laboratories with interest in the method, including regulatory agencies, industry, and universities.

Letter of Invitation

Address a formal letter to the individual responsible for assignment of laboratory effort. State reason for selecting that laboratory (e.g., as a volunteer or has responsibility or familiarity with the problem or method), estimated number of person-hours required for performance, number of test samples to be sent, number of analyses to be required, expected date for test sample distribution, and target date for completion of the study. *Emphasize the importance of management support in assigning the necessary time for the project*. Enclose a copy of the method and a return form or card (with postage affixed, if appropriate), requiring only a check mark for acceptance or refusal of the invitation, a signature, space for address corrections, telephone and fax numbers, e-mail, and date.

Laboratory Coordinator

With large studies, involving several analysts per laboratory, several familiarization samples, receipt of items at different times, or similar recurrent situations, acceptance of the invitation should be followed by a letter suggesting that a Laboratory Coordinator be appointed. The Laboratory Coordinator should be responsible for receiving and storing the study materials, assigning the work, dispensing study materials and information related to the study, seeing that the method is followed as written, accumulating the data, assuring that the data are correctly reported, and submitting the collaborative study manuscript within the deadline.

1.6 Instructions and Report Forms

Carefully design and prepare instructions and forms, and scrutinize them before distribution. A pilot study is also useful for uncovering problems in these documents.

Send instructions and report forms immediately on receipt of acceptance, independent of study materials, if selection of laboratories is not to be based on performance in pilot or training studies. The instructions should include in bold face or capital letters a statement:

THIS IS A STUDY OF THE METHOD, NOT OF THE LABORATORY. THE METHOD MUST BE FOLLOWED AS CLOSELY AS PRACTICABLE, AND ANY DEVIATIONS FROM THE METHOD AS DESCRIBED, NO MATTER HOW TRIVIAL THEY MAY SEEM, MUST BE NOTED ON THE REPORT FORM.

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Include instructions on storage and handling, markings, and identifications to be noted, any special preparation for analysis, and criteria for use of practice or familiarization samples, if included. Pre-code the form for each laboratory and provide sufficient space for as much sequential data as may be required for proper evaluation of the results, including a check of the calculations.

The initiating laboratory should indicate the number of significant figures to be reported, usually based on the output of the measuring instrument.

Note: In making statistical calculations from the reported data, the full power of the calculator or computer is to be used with no rounding or truncating until the final reported mean and standard deviations are achieved. At this point the standard deviations are rounded to 2 significant figures and the means and relative standard deviations are rounded to accommodate the significant figures of the standard deviation. For example, if the reproducibility standard deviation $s_R = 0.012$, the mean is reported as 0.147, not as 0.1473 or 0.15, and RSD_R, relative reproducibility standard deviation, is reported as 8.2%. If standard deviation calculations must be conducted manually in steps, with the transfer of intermediate results, the number of significant figures to be retained for squared numbers should be at least 2 times the number of figures in the data plus 1.

When recorder tracing reproductions are required to evaluate method performance, request their submission both in the instructions and as a check item on the form. Provide instructions with regard to labeling of recorder tracings, such as identification with respect to item analyzed, axes, date, submitter, experimental conditions, and instrument settings.

Include in the report form a signature line for the analyst and lines for a printed or typed version of the name and address for correct acknowledgement.

Provide for a review by the laboratory supervisor. An example of a completed form is helpful. A questionnaire may be included or sent after completion of the analyses in which the questions can be designed to reveal if modifications have been made at critical steps in the method.

Request a copy of the calibration curve or other relationship between response and concentration or amount of analyte so that if discrepancies become apparent after examining all of the data, it can be determined whether the problem is in the calibration or in the analysis.

1.7 Familiarization or Practice Samples

If deemed necessary, supply as far ahead as practicable, familiarization samples, with instructions, before actual materials are sent. When familiarization samples have been submitted, supply forms for reporting progress toward satisfactory performance.

2. Design of the Collaborative Study

2.1 General Principles

The purpose of a collaborative study is to determine estimates of the attributes of a method, particularly the "precision" of the method that may be expected when the method is used in actual practice. The AOACI uses 2 terms to define the precision of a method under 2 circumstances of replication: repeatability and reproducibility. Repeatability is a measure of the variation, s_r^2, between replicate determinations by the same analyst. It defines how well an analyst can check himself using the same method on blind replicates of the same material or split levels (Youden pairs), under the same conditions (e.g., same laboratory, same apparatus, and same time). Reproducibility is a composite measure of variation, s_R^2, which includes the between-laboratory and within-laboratory variations. It measures how well an analyst in a given laboratory can check the results of another analyst in another laboratory using the same method to analyze the same test material under different conditions (e.g., different apparatus and different time). The between-laboratory variation represents a systematic error that reflects variation arising from environmental conditions (e.g., condition of reagent and instruments, variation in calibration factors, and interpretations of the steps of the method) associated with the laboratories used in the study. Therefore, it is important to identify the causes of the differences among laboratories so that they may be controlled. Otherwise they will be summed into s_R^2.

Present test samples sent for analysis as unknowns (blind) and coded in a random pattern. If necessary to conserve analyst time, an indication of the potential range of concentration or amount of analyte may be provided. If spiking solutions are used, provide one coded solution for each material. All spiking solutions should be identical in appearance and volume. Do not provide a single solution from which aliquots are to be removed for spiking. Any information with regard to concentration (e.g., utilizing factorial aliquots or serial dilutions of the same spiking solutions) or known replication is likely to lead to an underestimate of the variability.

The study must be extensive enough to assure sufficient data surviving in the face of possible loss of materials during shipment, inability of collaborators to participate after acceptance, and a maximum outlier rate of 2/9 and still maintain valid data from a minimum of 8 laboratories.

Improper preparation of reference standards and standard solutions can cause a significant portion of the analytical error. A decision must be made whether such error is to be considered separately or as part of the method, i.e., will the analysts procure their own standard solutions or will standards be provided by the Study Director. The decision depends primarily on the availability of the standard. If the standard is readily available, the analysts should prepare their own. If the standard is not readily available, the standard may be supplied, but physical constants, e.g., absorptivity of working standard solutions, should be incorporated into the description as a check on proper preparation of the solution.

Obtain the necessary administrative and operational approvals. Review by potential users of the method is also desirable.

2.2 Laboratories

Laboratories must realize the importance of the study. A large investment is being made in studying the method and this probably will be only collaborative study of the method that will performed. Therefore, it is important to have a fair and thorough evaluation of the method.

Type

The most appropriate laboratory is one with a responsibility related to the analytical problem. Laboratory types may be representative (selection of laboratories that will be using the method in practice), reference (assumed to be "best"), or the entire population of laboratories (usually certified or accredited) that will be using the method. Final selection of participants should be based on a review with the General Referee and others of each laboratory's capabilities and past performance in collaborative studies, followed up, if possible, by telephone conversations or by personal visits. Selection may

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also be based on performance with familiarization samples. Sometimes only laboratories with dedicated or very specialized instruments must be used. If the study is intended for international consideration, laboratories from different countries should be invited to participate.

Number of Laboratories

Minimum of 8 laboratories submitting valid data (to avoid unduly large confidence bands about the estimated parameters). Only in special cases of very expensive equipment or specialized laboratories may the study be conducted with a minimum of 5 laboratories. Fewer laboratories widen the confidence limits of the mean and of the variance components (*see* design considerations). The optimum number of laboratories, balancing logistics and costs against information obtained, often is 8–10. However, larger studies are not discouraged.

For qualitative analyses, a minimum of 10 laboratories is needed; collaborative study must be designed to include 2 analyte levels per matrix, 6 test samples per level, and 6 negative controls per matrix. (*Note 1*: AOAC criteria for qualitative analyses are not part of the harmonized guidelines.)

Analysts

Most designs require only 1 analyst per laboratory. If analyst–within-laboratory variability is a desired variance component, multiple analysts should be requested from all participating laboratories. Ordinarily 2 analysts from the same laboratory cannot be substituted for different laboratories, unless standard solutions, reagents, chromatographic columns and/or materials, instrument calibrations, standard curves, etc., are prepared independently, and no consultation is permitted during the work. Different laboratories from the same organization may be used as separate laboratories if they operate independently with their own instruments, standards, reagents, and supervision.

2.3 Test Materials

Homogeneous Materials

Materials must be homogeneous; this is critical. Establish homogeneity by testing a representative number of laboratory samples taken at random before shipment. (A collaborator who reports an outlying value will frequently claim receipt of a defective laboratory sample.) The penalty for inhomogeneity is an increased variance in the analytical results that is not due to the intrinsic method variability.

Test Sample Coding

Code test samples at random so that there is no pre-selection from order of presentation.

Concentration Range

Choose analyte levels to cover concentration range of interest. If concentration range of interest is a tolerance limit or a specification level, bracket it and include it with materials of appropriate concentration. If design includes the determination of absence of analyte, include blank (not detectable) materials as part of range of interest.

Number of Materials

A minimum of 5 materials must be used in the collaborative study. Three materials are allowed but only when a single specification is involved for a single matrix.

Note 1: A material is an analyte (or test component)/matrix/concentration combination to which the method-performance parameters apply. This parameter determines the applicability of the method.

Note 2: The 2 test samples of blind or open duplicates are a single material (they are not independent).

The 2 test samples constituting a matched pair (called X and Y) are considered Youden matched pairs only if they are sufficiently close in composition. "Sufficiently close" would be considered as ≤5% difference in composition between X and Y. That is, given that the concentration of analyte in X (x_c) is higher than the concentration of the analyte in Y (y_c) then:

$$\frac{x_c \quad y_c}{x_c} \le 0.05$$

or:

$$y_c \ge (x_c - 0.05x_c)$$

Note 3: The blank or negative control may or may not be a material, depending on the usual purpose of the analysis. For example, in trace analysis, where very low levels (near the limit of quantitation) are often sought, the blanks are considered as materials, and are necessary to determine certain statistical "limits of measurement;" however, if the blank is merely a procedural control, in macro-level analysis (e.g., fat in cheese), it would not be considered a material.

Nature of Materials

Materials should be representative of commodities usually analyzed, with customary and extreme values for the analyte.

Size of Test Samples

Furnish only enough test sample to provide the number of test portions specified in the instructions. If additional test portions are required, the collaborator must request them, with an explanation.

Interferences

If pertinent, some materials, but not all, should contain contaminants and interferences in concentrations likely to be encountered, unless they have been shown to be unimportant through within-laboratory study. The success of the method in handling interference on an intralaboratory basis will be demonstrated by passing systems suitability tests.

Familiarization Samples

With new, complex, or unfamiliar techniques, provide material(s) of stated composition for practice, on different days, if possible. The valuable collaborative materials should not be used until the analyst can reproduce the stated value of the familiarization samples within a given range. However, it should be pointed out that one of the assumptions of analysis of variance is that the underlying distribution

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of results is independent of time (i.e., there is no drift). The Study Director must be satisfied that this assumption is met.

2.4 Replication

When within-laboratory variability is also of interest, as is usually the case, independent replication can be ensured by applying at least one of the following procedures (listed in suggested order of desirability; the nature of the design should not be announced beforehand):

(*1*) *Split levels (Youden pairs)*.—The 2 test materials, nearly identical but of slightly different composition (e.g., ≤5% difference in composition, *see 2.3 Number of Materials, Note 2*) are obtained either naturally or by diluting (or by fortifying) one portion of the material with a small amount of diluent (or of analyte). Both portions are supplied to the participating laboratories as test samples, each under a random code number, and each test sample should be analyzed only once; replication defeats the purpose of the design.

(*2*) *Split levels for some materials and blind duplicates for other materials in the same study*.—Obtain only single values from each test sample supplied.

(*3*) *Blind duplicate test samples, randomly coded*.—*Note*: Triplicate and higher replication are relatively inefficient when compared with duplicate test samples because replication provides additional information only on individual within-laboratory variability, which is usually the less important component of error. It is more effective to utilize resources for the analysis of more levels and/or materials rather than for increasing the number of replicates for the individual materials.

PRACTICAL PRINCIPLE: With respect to replication, the greatest net marginal gain is always obtained in going from 2 to 3 as compared to going from 3 to 4, 4 to 5, etc.

(*4*) *Independent materials*.—(*Note*: Unrelated independent materials may be used as a split level in the calculations of the precision parameters or for plotting. There should be ≤5% difference in composition for such materials (*see 2.3 Number of Materials, Note 2*). The more they differ in concentration, the less reliable the information they provide on within-laboratory variability.)

(*5*) *Known replicates*.—*Use of known replicates is a common practice*.—It is much preferable to use the same resources on blind replicates or split levels.

(*6*) *Quality control materials*.—Instead of obtaining repeatability parameters through the collaborative study, information can be obtained from use of quality control materials in each laboratory individually, for its own use, independent of the collaborative study, for a separate calculation of s_r, using 2 (or more) replicates from each quality control test, according to the pattern developed for each product.

2.5 Other Design Considerations

The design can be reduced in the direction of less work and less cost, but at the sacrifice of reduced confidence in the reliability of the developed information.

More work (values) is required if more confidence is needed, e.g., greater confidence is required to enforce a tolerance at 1.00 mg/kg than at 1.0 mg/kg. (The distinction is a precision requirement of the order of 1% rather than 10%.)

The estimate of the standard deviation or the corresponding relative standard deviation obtained from a collaborative study is a random variable that varies about its corresponding true value. For example, the standard deviation, s_r, which measures within laboratory or repeatability precision has associated with it a standard deviation (STD = s_r) describing its scatter about the true value σ_r. Therefore, s_r, whose STD (s_r) is a function of s_r^2, number of laboratories, and number of analyses per laboratory, will vary about σ_r from occasion-to-occasion even for the same test conditions and material. The STD s_R, which measures among laboratory or reproducibility precision, has a STD (s_R) that is a function of the random variables s_r^2 and s_L^2, number of laboratories, and number of analyses per laboratory. s_R will vary about its true value σ_R from occasion-to-occasion for the same test material.

The validity of extrapolating the use of a method beyond concentrations and components tested can be estimated only on the basis of the slope of the calibration curve (sensitivity) observed as a function of the nature and concentration of the matrix and contaminant components. If the signal is more or less independent of these variables, a reasonable amount of extrapolation may be utilized. The extrapolator assumes the burden of proof as to what is reasonable.

3. Preparation of Materials for Collaborative Studies

3.1 General Principles

Heterogeneity between test samples from a single test material must be negligible compared to analytical variability, as measured within the Study Director's laboratory.

The containers must not contribute extraneous analytes to the contents, and they must not adsorb or absorb analytes or other components from the matrix, e.g., water.

If necessary, the materials may be stabilized, preferably by physical means (freezing, dehydrating), or by chemical means (preservatives, antioxidants) which do not affect the performance of the method.

Composition changes must be avoided, where necessary, by the use of vapor-tight containers, refrigeration, flushing with an inert gas, or other protective packaging.

3.2 Materials Suitable for Collaborative Studies

Material and analyte stability: Ensure analyte and matrix stability over projected transport and projected length of study.

Single batch of homogenous, stable product such as milk powder, peanut butter, vegetable oil, starch, etc., is the best type of material.

Reference materials supplied by standards organizations such as National Institute of Standards and Technology (NIST, Gaithersburg, MD) and EC's Joint Research Center and Institute on Reference Materials and Methods (IRMM, Belgium) are excellent, unless they have easily recognizable characteristics (e.g., odor and color of NIST Orchard Leaves). However, they are of limited availability, composition, and analyte level. If available, they are expensive. Sometimes the certification organization may be interested in making reference materials available for the analyte under study, in which case it may assist in providing the material for the study.

Synthetic materials may be especially formulated with known amounts of analytes by actual preparation for the study. This procedure is best used for macro-constituents such as drugs or pesticide formulations.

Spiked materials consisting of normal or blank materials to which a known amount of analyte has been added may be used. The amount of analyte added should not be excessive in relation to the amount present (e.g., about 2×), and the analyte added should be in the same

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chemical form as present in the commodities to be analyzed subsequently.

In drug and pesticide residue-type problems, it is often necessary to use spiked materials in order to assess recovery. However, because incurred residues are likely to present different problems from those of spiked residues, collaborative studies should include some test samples with incurred residues to ensure that the method is applicable under these conditions as well.

(*1*) *Preparation in bulk*.—This requires thorough and uniform incorporation of analyte, often by serial dilution of solids. The danger of segregation due to differences in densities always exists. Fluid materials susceptible to segregation should be prepared under constant agitation. Uniformity should be checked by direct analysis, with an internal standard, or by a marker compound (dye or radioactive label).

(*2*) *Test samples, individually prepared*.—A known amount of analyte is either weighed directly or added as an aliquot of a prepared solution to pre-measured portions of the matrix in individual containers. The collaborator is instructed to use each entire portion for the analysis, transferring the contents of the container quantitatively or a substantial weighed fraction of the portion. (This is the preferred alternative to spiked solid materials at trace [mg/kg] levels, at the expense of considerably more work.)

(*3*) *Concentrated unknown solutions for direct addition by collaborators to their own commodities*.—Should be used only as a last resort when instability of the analyte precludes distribution from a central point. To preclude direct analysis of the spiking solution, supply individual coded solutions to be added in their entirety to portions of the matrix for single analyses by each laboratory. All solutions should have the same volume and appearance. This type of material is analogous to that of test samples except for the source of matrix. This case should be used only for perishable commodities that are altered by all available preservation techniques.

Materials analyzed by another, presumably accurate, method, if available, in the Study Director's laboratory or by some or all the collaborators.

Only as an absolutely last resort (usually with unstable materials and preparation of material studies) should the collaborators be permitted to prepare their own materials for analysis. Since it is impossible to avoid the personal bias introduced by knowledge of the composition of the material, the materials should be prepared in each laboratory by an individual who will not be involved in the analyses.

3.3 Blanks

When the absence of a component is as important as its presence, when determinations must be corrected for the amount of the component or the presence of background in the matrix, or when recovery data are required, provision must be made for the inclusion of blank materials containing "none" (not detected) of the analyte. It is also important to know the variability of the blank and the tendency of the method to produce false positives. There are 2 types of blanks: matrix blanks and reagent blanks. Since laboratories often will utilize reagents from different sources, each laboratory should perform reagent blanks. Matrix blanks, when required, are an intrinsic part of the method, and the number of blanks needed depends on the combined variance of the material (s_M) and of the blank (s_B). Standard deviation reflecting the total variability of a blank corrected value will be $s = (s_M^2 + s_B^2)^{1/2}$.

3.4 Limit of Detection/Quantitation

If the limit of detection/quantitation is important, it is necessary to provide a design which gives special attention to the number of blanks, and to the necessity for interpreting false positives and false negatives. In all cases, the definition of limit of detection/quantitation used in the study must be given by the Study Director.

3.5 Controls

When separation from interferences is critical to the analysis, appropriate materials incorporating these interferences must be included.

PRACTICAL ADVICE: Always allow for contingencies and prepare more sets (e.g., 25% more) of laboratory samples than there are collaborators. Some packages may never arrive, some materials may spoil, and some may be lost or the container broken. New laboratories may have to be substituted for those which are unable to complete the promised work. Some sets may have to be analyzed at a later time for different purposes, such as to verify stability on storage.

4. Submission of Test Samples

4.1 Sending Collaborative Study Material

Notify collaborators of shipping arrangements, including waybill numbers, arrival time, and required storage conditions.

Label test samples legibly and without ambiguity.

Pack shipping cartons well and label properly to avoid transportation delays. If the containers are breakable, pack well to minimize possibility of breakage. If material is perishable, ship frozen with solid CO_2, sufficient to last several days longer than anticipated travel time. Use special transportation services, if necessary. For international delivery, mark as "Laboratory samples—no commercial value" or other designation as required by customs regulations of the country to which the package is being sent. Hazardous materials must be packed and labeled as required by transportation regulations. Animal and plant products sent across international borders may require special certification from health authorities.

Include a return slip, to confirm safe receipt, with each package. If not sent previously, include copy of method, instructions, and report forms.

Provide instructions for proper storage of test samples between unpacking and analysis. Note that analysts should not use thawed or decomposed test samples without consulting the Study Director.

When it is important to have instruments calibrated with the same reference material, supply reference material to collaborators. Provision for supplying reference standards is particularly important when commercial sources of standards have not yet been developed. The inclusion of a working standard solution as an unknown is useful to establish a consensus value for standardization of quality control parameters, such as absorptivity, retention time, and sensitivity (change in signal intensity divided by the change in concentration).

4.2 Obligations of Collaborators

Analyze test samples at times indicated, according to submitted protocol. With unstable materials (e.g., with microbial or decomposition problems), analyses must be started at specified times.

FOLLOW METHOD EXACTLY (*this is critical*). If method is unclear, contact Study Director. Any deviation, such as the necessity to substitute reagents, columns, apparatus, or instruments, must be

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recorded at the time and reported. If the collaborator has no intention of following the submitted method, he or she should not participate in the study. If the collaborator wishes to check another method on the same materials, additional test samples should be requested for that purpose, to be analyzed separately.

Conduct exactly the number of determinations stated in the instructions. Any other number complicates the statistical analysis. Too few determinations may require discarding the results from that laboratory for that material or inserting "missing values"; too many values may require discarding the contribution of that laboratory or at least some of the values. If a laboratory cannot follow instructions as to number of analyses to perform, it raises a question as to its ability to follow the method.

Report individual values, including blanks. Do not average or do other data manipulations unless required by the instructions. Undisclosed averaging distorts statistical measures. If blank is larger than determination, report the negative value; do not equate negative values to zero. Follow or request instructions with regard to reporting "traces" or "less than." Descriptive (i.e., nonquantitative) terms are not amenable to statistical analysis and should be avoided. When results are below the limit of determination, report actual calculated result, regardless of its value.

Supply raw data, graphs, recorder tracings, photographs, or other documentation as requested in the instructions.

Since collaborators may have no basis for judging whether a value is an outlier, the results should be communicated to the Study Director as soon as the protocol is complete and before time and equipment are reassigned, so that repeat assays may be performed at once, if necessary and if permitted by the protocol.

Note: The sooner an apparent outlier is investigated, the greater the likelihood of finding a reason for its occurrence.

The most frequent causes of correctable outliers are:

- Incorrect calculations and arithmetic errors.
- Errors in reporting, such as transposition of numbers, misplacement of the decimal point, or use of the wrong units.
- Incorrect standards due to weighing or volumetric errors (check physical constants or compare against freshly prepared standard solutions).
- Contamination of reagents, equipment, or test samples.

5. Statistical Analysis

5.1 Initial Review of Data (Data Audit)

The Study Director may first plot the collaborative study results, material by material (or one value against the other for a split level [Youden pair]), value vs laboratory, preferably in ascending or descending order of reported average concentration. Usually major discrepancies will be apparent: displaced means, unduly spread replicates, outlying values, differences between methods, consistently high or low laboratory rankings, etc.

Only valid data should be included in the statistical analysis. Valid data are values that the Study Director has no reason to suspect as being wrong. Invalid data may result when: (*1*) the method is not followed; (*2*) a nonlinear calibration curve is found although a linear curve is expected; (*3*) system suitability specifications were not met; (*4*) resolution is inadequate; (*5*) distorted absorption curves arise; (*6*) unexpected reactions occur; or (*7*) other atypical phenomena materialize. Other potential causes of invalid data are noted previously.

5.2 Outliers

Collaborative studies seem to have an inherent level of outliers, the number depending on the definition of outliers and the basis for calculation (analytes, materials, laboratories, or determinations). Rejection of more than 2/9 of the data from each material in a study, without an explanation (e.g., failure to follow the method), is ordinarily considered excessive. Study must maintain valid data from a minimum of 8 labs. For larger studies, a smaller acceptable percentage of rejections may be more appropriate. Determine the probability that the apparent aberrant value(s) is part of the main group of values considered as a normal population by applying the following tests in order:

(*1*) *Cochran test* for removal of laboratories (or indirectly for removal of extreme individual values from a set of laboratory values) showing significantly greater variability among replicate (within-laboratory) analyses than the other laboratories for a given material. Apply as a 1-tail test at a probability value of 2.5%.

To calculate the Cochran test statistic: Compute the within-laboratory variance for each laboratory and divide the largest of these by the sum of all of these variances. The resulting quotient is the Cochran statistic which indicates the presence of a removable outlier if this quotient exceeds the critical value listed in the Cochran table for P = 2.5% (1-tail) and L (number of laboratories), **Appendix 1**.

(*2*) Grubbs tests for removal of laboratories with extreme averages. Apply in the following order: single value test (2-tail; P = 2.5%); then if no outlier is found, apply pair value test (2 values at the highest end, 2 values at the lowest end, and 2 values, one at each end, at an overall P = 2.5%).

To calculate the single Grubbs test statistic: Compute the average for each laboratory and then calculate the standard deviation (SD) of these L averages (designate as the original s). Calculate the SD of the set of averages with the highest average removed (s_H); calculate the SD of the set averages with the lowest average removed (s_L). Then calculate the percentage decrease in SD as follows:

$$100 \times [1 - (s_L/s)] \text{ and } 100 \times [1 - (s_H/s)]$$

The higher of these 2 percentage decreases is the single Grubbs statistic, which signals the presence of an outlier to be omitted if it *exceeds* the critical value listed in the single Grubbs tables at the P = 2.5% level, 2-tail, for L laboratories, **Appendix 2**.

To calculate the Grubbs pair statistic, proceed in an analogous fashion, except calculate the standard deviations s_{2L}, s_{2H}, and s_{HL}, following removal of the 2 lowest, the 2 highest, and the highest and the lowest averages, respectively, from the original set of averages. Take the smallest of these 3 SD values and calculate the corresponding percentage decrease in SD from the original s. A Grubbs outlier pair is present if the selected value for the percentage decrease from the original s *exceeds* the critical value listed in the Grubbs pair value table at the P = 2.5% level, for L laboratories, **Appendix 2**.

(*3*) If the single value Grubbs test signals the need for outlier removal, remove the single Grubbs outlier and recycle back to the Cochran test as shown in the flow chart, **Appendix 3**.

If the single value Grubbs test is negative, check for masking by performing the pair value Grubbs test. If this second test is positive,

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remove the 2 values responsible for activating the test and recycle back to the Cochran test as shown in the flow chart, **Appendix 3**, and repeat the sequence of Cochran, single value Grubbs, and pair value Grubbs. Note, however, that outlier removal should stop before more than 2/9 laboratories are removed.

(*4*) If no outliers are removed for a given cycle (Cochran, single Grubbs, pair Grubbs), outlier removal is complete. Also, stop outlier removal whenever more than 2/9 of the laboratories are flagged for removal. With a higher removal rate, either the precision parameters must be taken without removal of all outliers or the method must be considered as suspect.

Note: The decision as to whether a value(s) should be removed as an outlier ultimately is not statistical in nature. The decision must be made by the Study Director on the basis of the indicated probability given by the outlier test and any other information that is pertinent. (However, for consistency with other organizations adhering to the harmonized outlier removal procedure, the estimate resulting from rigid adherence to the prescribed procedure should be reported.)

5.3 Bias (Systematic Deviation) of Individual Results

Bias is defined as follows:

(Estimated) bias =
mean amount found – amount added (or known or assigned value)

Single-value error and recovery are defined as follows:

Error of a single value =
the single value – amount added (true value)

There are 2 methods for defining percent recovery: marginal and total. The formulas used to estimate these percent recoveries are provided in the following:

$$\text{Marginal \%Rec} = 100R_M = 100((C_f - C_u)/C_A)$$

$$\text{Total \%Rec} = 100R_T = 100(C_f)/(C_u + C_A)$$

where C_f is the amount found for the fortified concentration, C_u is the amount present originally for the unfortified concentration, and C_A is the amount added for the added concentration. The amount added is known or fixed and should be a substantial fraction of, or more than, the amount present in the unfortified material; all other quantities are measured and are usually reported as means, all of which have variations or uncertainties. The variation associated with the marginal percent recovery is $\mathrm{var}(100R_M) = (100^2/C_A^2)[\mathrm{var}(C_f) + \mathrm{var}(C_u)]$ is larger than the variation associated with the total percent recovery. The variation associated with total percent recovery is $\mathrm{var}(100R_T) = [100^2/(C_u + C_A)^2][\mathrm{var}(C_f) + (R_T^2)\mathrm{var}(C_u)]$. In each formula var means variance and refers to the concentration variation for the defined concentrations.

A true or assigned value is known only in cases of spiked or fortified materials, certified reference materials, or by analysis by another (presumably unbiased) method. Concentration in the unfortified material is obtained by direct analysis by the method of additions. In other cases, there is no direct measure of bias, and consensus values derived from the collaborative study itself often must be used for the reference point.

Notes: (*1*) Youden equates "true" or "pure" between-laboratory variability (not including the within-laboratory variability) to the variability in bias (or variability in systematic error) of the individual laboratories. Technically, this definition refers to the average squared difference between individual laboratory biases and the mean bias of the assay.

(*2*) The presence of random error limits the ability to estimate the systematic error. To detect the systematic error of a single laboratory when the magnitude of such error is comparable to that laboratory's random error, at least 15 values are needed, under reasonable confidence limit assumptions.

5.4 Precision

The precision of analytical methods is usually characterized for 2 circumstances of replication: within laboratory or repeatability and among laboratories or reproducibility. Repeatability is a measure of how well an analyst in a given laboratory can check himself using the same analytical method to analyze the same test sample at the same time. Reproducibility is a measure of how well an analyst in one laboratory can check the results of another analyst in another laboratory using the same analytical method to analyze the same test sample at the same or different time. Given that test samples meet the criteria for a single material, the repeatability standard deviation (s_r) is:

$$s_r = (\Sigma d_i^2/2L)^{1/2}$$

where d_i is the difference between the individual values for the pair in laboratory i and L is the number of laboratories or number of pairs.

The reproducibility standard deviation (s_R) is computed as:

$$s_R = (1/2(s_d^2 + s_r^2))^{1/2}$$

where $s_d^2 = \Sigma(T_i - \overline{T})^2/(2(L-1))$, T_i is the sum of the individual values for the pair in laboratory i, $\overline{T}$ is the mean of the T_i across all laboratories or pairs, L is the number of laboratories or pairs, and s_r^2 is the square of $s_r = (\Sigma d_i^2/2L)^{1/2}$.

When the pairs of test samples meet the criteria for Youden matched pairs, i.e., when:

$$[(x_c - y_c)/x_c] \leq 0.05$$

or

$$y_c \geq (x_c - 0.05x_c),$$

s_r, a practical approximation for repeatability standard deviation, is calculated as:

$$s_r = [\Sigma(d_i - \overline{d})^2/(2(L-1))]^{1/2}$$

where d_i is the difference between the individual values for the pair in laboratory i, $\overline{d}$ is the mean of the d_i across all laboratories or pairs, and L is the number of laboratories or pairs. The reproducibility standard deviation, s_R, which reflects the square root of the average of the reproducibility variances for the individual materials (i.e., $s_R = [½(s_{Rx}^2 + s_{Ry}^2)]^{1/2}$), previously called X and Y, should be determined only if the individual variances are not significantly different from each other. To compare s_{Rx}^2 and s_{Ry}^2, the following formula may be used.

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$$t = \frac{(s^2_{Rx} \quad s^2_{Ry})(L \quad 2)^{1/2}}{2[(s^2_{Rx})(s^2_{Ry}) \quad (cov_{xy})^2]^{1/2}}$$

where $s_{Rx}{}^2 = [1/(L-1)][\Sigma x_i{}^2 - (\Sigma x_i)^2/L]$, $s_{Ry}{}^2 = [1/(L-1)][\Sigma y_i{}^2 - (\Sigma y_i)^2/L]$, and $cov_{xy} = [1/(L-1)][\Sigma x_i y_i - (\Sigma x_i \Sigma y_i)/L]$. If t is greater than or equal to the tabular t-value for L – 2 degrees of freedom for a significance level of $\alpha = 0.05$, this may be taken to indicate that $s_{Rx}{}^2$ and $s_{Ry}{}^2$ are not equivalent and should not be pooled for a single estimate of $s_R{}^2$. That is, $s_{Rx}{}^2$ and $s_{Ry}{}^2$ should be taken as the reproducibility variance estimates for the individual test materials X and Y, respectively. This means that there is no rigorous basis for calculating $s_r{}^2$ because the within laboratory variability cannot be estimated directly.

Though s_r and s_R are the most important types of precision, it is the relative standard deviations (RSD_r % = $100s_r$/mean and RSD_R % = $100s_R$/mean) that are the most useful measures of precision in chemical analytical work because the RSD values are usually independent of concentration. Therefore, the use of the RSD values facilitates comparison of variabilities at different concentrations. When the RSD increases rapidly with decreasing concentration or amount, the rise delineates the limit of usefulness of the method (limit of reliable measurement).

5.5 HORRAT

HORRAT value is the ratio of the reproducibility relative standard deviation, expressed as a percent (RSD_R, %) to the predicted reproducibility relative standard deviation, expressed as a percent ($PRSD_R$, %), i.e.,

$$\text{HORRAT} = \frac{RSD_R, \%}{PRSD_R, \%}$$

where $PRSD_R$, % = $2C^{-0.1505}$ and C = the estimated mean concentration. HORRAT values between 0.5 to 1.5 may be taken to indicate that the performance value for the method corresponds to historical performance. The limits for performance acceptability are 0.5–2.

The precision of a method must be presented in the collaborative study manuscript. The HORRAT will be used as a guide to determine the acceptability of the precision of a method.

The HORRAT is applicable to most chemical methods. HORRAT is not applicable to physical properties (viscosity, RI, density, pH, absorbance, etc.) and empirical methods [e.g., fiber, enzymes, moisture, methods with indefinite analytes (e.g., polymers) and "quality" measurements, e.g., drained weight]. Deviations may also occur at both extremes of the concentration scale (near 100% and $\leq 10^{-8}$). In areas where there is a question if the HORRAT is applicable, the General Referee will be the determining judge.

The following guidelines should be used to evaluate the assay precision:

- HORRAT ≤ 0.5—Method reproducibility may be in question due to lack of study independence, unreported averaging, or consultations.
- 0.5 < HORRAT ≤ 1.5—Method reproducibility as normally would be expected.
- HORRAT > 1.5—Method reproducibility higher than normally expected: the Study Director should critically look into possible reasons for a "high" HORRAT (e.g., were test samples sufficiently homogeneous, indefinite analyte or property?), and discuss this in the collaborative study report.
- HORRAT > 2.0—Method reproducibility is problematic. A high HORRAT may result in rejection of a method because it may indicate unacceptable weaknesses in the method or the study. Some organizations may use information about the HORRAT as a criterion not to accept the method for official purposes (e.g., this is currently the case in the EU for aflatoxin methods for food analysis, where only methods officially allowed are those with HORRATs ≤ 2).

5.6 Incorrect, Improper, or Illusory Values (False Positive and False Negative Values)

These results are not necessarily outliers (no *a priori* basis for decision), since there is a basis for determining their incorrectness (a positive value on a blank material, or a zero (not found) or negative value on a spiked material). There is a statistical basis for the presence of false negative values: In a series of materials with decreasing analyte concentration, as the RSD increases, the percent false negatives increases from an expected 2% at an RSD = 50% to 17% at an RSD = 100%, merely from normal distribution statistics alone.

When false positives and/or false negatives exceed about 10% of all values, analyses become uninterpretable from lack of confidence in the presence or absence of the analyte, unless all positive laboratory samples are re-analyzed by a more reliable (confirmatory) method with a lower limit of determination than the method under study. When the proportion of zeros (not necessarily false negatives) becomes greater than approximately 30%, the distribution can become bimodal and even more uninterpretable (is the analyte present or absent?).

5.7 Final Collaborative Study Manuscript

The final manuscript should contain a description of the materials used, their preparation, any unusual features in their distribution, and a table of all *valid* data, including outliers. When replication is performed, the individual values, not just averages, must be given, unless the method requires averages (e.g., microbiological methods). Values not used for specified reasons, such as decomposition, failure to follow method, or contamination, should not be included in the table since they may be included erroneously in subsequent recalculations. AOAC INTERNATIONAL requires the calculation and reporting of mean, percent recovery (% Rec), HORRAT, repeatability (within-laboratory, s_r) and reproducibility (interlaboratory, s_R) standard deviations, and repeatability and reproducibility relative standard deviations (RSD_r and RSD_R, respectively). The accuracy (bias, trueness) of a method measuring a specific, identifiable analyte should be presented in the collaborative study manuscript as a recovery of added (spiked) analyte, as the results of analysis of a reference material, or by comparison with results by a reference method. Methods that are unable to report accuracy because of the unavailability of an accepted "true" value, or because of the nature of the method (empirical, microbiological, quality factors) should mention the reason in the manuscript. Proofread tables very carefully since many errors are of typographical origin. Give the names of the participants and their organizations, including complete contact information (name, preliminary address, telephone and fax numbers, and e-mail address).

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The final manuscript should be published in a generally accessible publication, or availability of the report from the organization sponsoring the method should be indicated in the published method. Without public documentation, the significance of the study is very limited.

The manuscript should be sent to all participants, preferably at the preliminary stage, so that clerical and typographical errors may be corrected before publication. If changes in values from the original submission are offered, they must be accompanied by an explanation.

Example of Table of Interlaboratory Study Results: *See* **Table 1**.

The summary table as it will appear in the *Official Methods of Analysis of AOAC INTERNATIONAL* is given in **Table 2**.

6. References

(1) W.J. Youden & E.H. Steiner (1975) *Statistical Manual of the AOAC*, AOAC INTERNATIONAL, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-7077, USA. The fifth printing (1987) contains several explanatory footnotes.

(2) G.T. Wernimont (1985) *Use of Statistics to Develop and Evaluate Analytical Methods*, W. Spendley (Ed.) AOAC INTERNATIONAL, 481 N. Frederick Ave, Suite 500, Gaithersburg, MD 20877-7077, USA.

(3) T. Dols & B. Armbrecht (1976) *J. Assoc. Off. Anal. Chem*. **59**, 1204–1207.

(4) International Organization for Standardization Guide 18, ISO, Case Postale 56, CH-1211 Geneva, Switzerland, and other national standards organizations.

(5) International Organization for Standardization ISO 5725, ISO, Case Postale 56, CH-1211 Geneva, Switzerland, and other national standards organizations.

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Appendix 1 Critical values for the Cochran maximum variance ratio at the 2.5% (1-tail) rejection level, expressed as the percentage the highest variance is of the total variance

L = number of laboratories at a given level (concentration)
r = number of replicates per laboratory

L	r = 2	r = 3	r = 4	r = 5	r = 6
4	94.3	81.0	72.5	65.4	62.5
5	88.6	72.6	64.6	58.1	53.9
6	83.2	65.8	58.3	52.2	47.3
7	78.2	60.2	52.2	47.3	42.3
8	73.6	55.6	47.4	43.0	38.5
9	69.3	51.8	43.3	39.3	35.3
10	65.5	48.6	39.9	36.2	32.6
11	62.2	45.8	37.2	33.6	30.3
12	59.2	43.1	35.0	31.3	28.3
13	56.4	40.5	33.2	29.2	26.5
14	53.8	38.3	31.5	27.3	25.0
15	51.5	36.4	29.9	25.7	23.7
16	49.5	34.7	28.4	24.4	22.0
17	47.8	33.2	27.1	23.3	21.2
18	46.0	31.8	25.9	22.4	20.4
19	44.3	30.5	24.8	21.5	19.5
20	42.8	29.3	23.8	20.7	18.7
21	41.5	28.2	22.9	19.9	18.0
22	40.3	27.2	22.0	19.2	17.3
23	39.1	26.3	21.2	18.5	16.6
24	37.9	25.5	20.5	17.8	16.0
25	36.7	24.8	19.9	17.2	15.5
26	35.5	24.1	19.3	16.6	15.0
27	34.5	23.4	18.7	16.1	14.5
28	33.7	22.7	18.1	15.7	14.1
29	33.1	22.1	17.5	15.3	13.7
30	32.5	21.6	16.9	14.9	13.3
35	29.3	19.5	15.3	12.9	11.6
40	26.0	17.0	13.5	11.6	10.2
50	21.6	14.3	11.4	9.7	8.6

Cochran statistic = (largest individual within-laboratory variance)/(sum of all the within-laboratory variances).

Appendix 2 Critical values for the Grubbs extreme deviation outlier tests at the 2.5% (2-tail), 1.25% (1-tail) rejection level, expressed as the percent reduction in the standard deviations caused by removal of the suspect value(s) (*see* text for calculating the Grubbs statistics)

L = number of laboratories at a given level (concentration)

L	One highest or lowest	Two highest or two lowest	One highest and one lowest
4	86.1	98.9	99.1
5	73.5	90.3	92.7
6	64.0	81.3	84.0
7	57.0	73.1	76.2
8	51.4	66.5	69.6
9	46.8	61.0	64.1
10	42.8	56.4	59.5
11	39.3	52.5	55.5
12	36.1	48.5	51.6
13	33.8	46.1	49.1
14	31.7	43.5	46.5
15	29.9	41.2	44.1
16	28.3	39.2	42.0
17	26.9	37.4	40.1
18	25.7	35.9	38.4
19	24.6	34.5	36.9
20	23.6	33.2	35.4
21	22.7	31.9	34.0
22	21.9	30.7	32.8
23	21.2	29.7	31.8
24	20.5	28.8	30.8
25	19.8	28.0	29.8
26	19.1	27.1	28.9
27	18.4	26.2	28.1
28	17.8	25.4	27.3
29	17.4	24.7	26.6
30	17.1	24.1	26.0
40	13.3	19.1	20.5
50	11.1	16.2	17.3

Source: Both tables were calculated by R. Albert (October 1993) by computer simulation involving several runs of approximately 7000 cycles each for each value, and then smoothed. Although the table of **Appendix 1** is strictly applicable only to a balanced design (same number of replicates from all laboratories), it can be applied to an unbalanced design without too much error, if there are only a few deviations.

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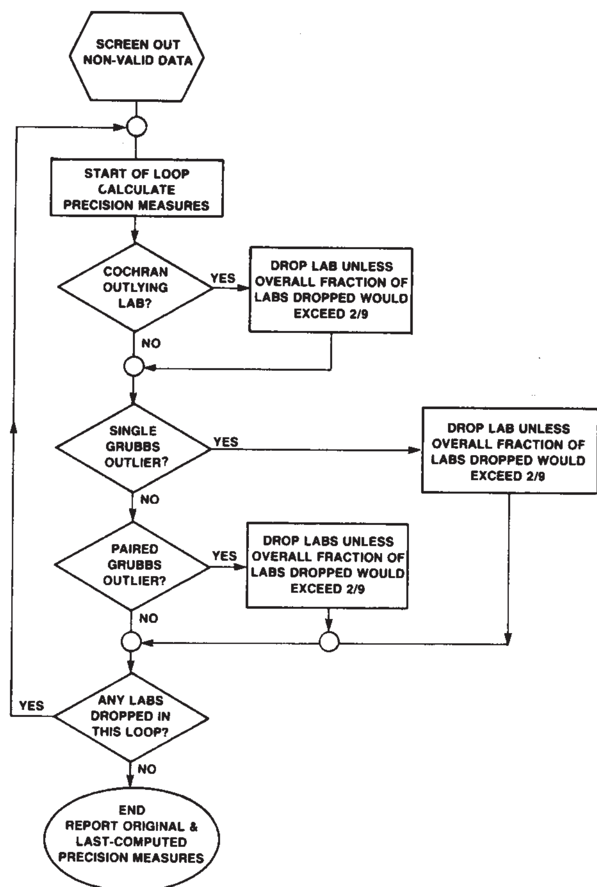

Appendix 3—Flowchart.

Table 1 [x] Collaborative tests carried out at the international level in [year(s)] by [organization(s)] in which [y and z] laboratories participated, each performing [k] replicates, gave the following statistical results [results expressed in (units)]:

Material [description and listed across the top in increasing order of magnitude of means]

Number of laboratories retained after eliminating outliers
Number of outlying laboratories removed

Mean ($\bar{}$)
True or accepted value, if known

Repeatability standard deviation (s_r)
Repeatability relative standard deviation (RSD_r)
Repeatability value, r ($2.8 \times s_r$)
Total within laboratory standard deviation (s_e)—optional if s_r is not valid.

Reproducibility standard deviation (s_R)
Reproducibility relative standard deviation (RSD_R)
HORRAT
Reproducibility value, R ($2.8 \times s_R$)
Percent recovery (% Rec), if applicable

The repeatability and reproducibility values may also be expressed as a relative value (as a percentage of the determined mean value), when the results so suggest.
If the recovery and precision values are more or less constant for all materials or for group of materials, an overall average value may be presented. Although such averaging may not have statistical validity, it does have practical value.

Table 2 Model table for presentation of chemistry results from AOAC Official Methods

Table 200X.XX Interlaboratory results for [analyte] by [technique]

Material						Reproducibility	
Matrix	Level (units)	No. of labs[a(b)]	Mean (units)	Recovery, %	Repeatabiltiy RSD_r, %	RSD_R, %	HORRAT

[a(b)] a = Number of laboratories remaining after removal of the number of outliers indicated by (*b*).

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Appendix B Continued: Methods Recommended by the Cannabis Science Task Force

Heavy metals

- METHOD 6020B: Inductively Coupled Plasma- Mass Spectrometry
- METHOD 6010D: Inductively Coupled Plasma - Optical Emissions Spectrometry
- METHOD 3050B, REVISION 2: Acid Digestion Of Sediments, Sludges, And Soils Method 3052: Microwave Assisted Acid Digestion Of Siliceous And Organically Based Matrices
- METHOD 3031: Acid Digestion Of Oils For Metals Analysis By Atomic Absorption Or Icp Spectrometry

METHOD 6020B

INDUCTIVELY COUPLED PLASMA—MASS SPECTROMETRY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute quality control (QC) acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of sub-µg/L concentrations of a large number of elements in water samples and in waste extracts or digests (Refs. 1 and 2). When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, and other solid wastes for which total (acid-leachable) elements are required. The analyst should insure that a sample digestion method is chosen that is appropriate for each analyte and the intended use of the data. Refer to Chapter Three for the appropriate digestion procedures.

1.2 ICP-MS has been applied to the determination of over 60 elements in various matrices. Analytes for which the acceptability of Method 6020 has been demonstrated through multi-laboratory testing on solid and aqueous wastes are listed below.

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Aluminum	Al	7429-90-5	Magnesium	Mg	7439-95-4
Antimony	Sb	7440-36-0	Manganese	Mn	7439-96-5
Arsenic	As	7440-38-2	Mercury	Hg	7439-97-6
Barium	Ba	7440-39-3	Nickel	Ni	7440-02-0
Beryllium	Be	7440-41-7	Potassium	K	7440-09-7

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Cadmium	Cd	7440-43-9	Selenium	Se	7782-49-2
Calcium	Ca	7440-70-2	Silver	Ag	7440-22-4
Chromium	Cr	7440-47-3	Sodium	Na	7440-23-5
Cobalt	Co	7440-48-4	Thallium	Tl	7440-28-0
Copper	Cu	7440-50-8	Vanadium	V	7440-62-2
Iron	Fe	7439-89-6	Zinc	Zn	7440-66-6
Lead	Pb	7439-92-1			

[a]Chemical Abstract Service Registry Number

The performance acceptability of ICP-MS for the determination of the listed elements was based upon comparison of the multi-laboratory testing results with those obtained from either furnace atomic absorption spectrophotometry or inductively coupled plasma—optical emission spectrometry. It should be noted that one multi-laboratory study was conducted in 1988. As advances in ICP-MS instrumentation and software have been made since that time, other elements have been added through validation and with additional improvements in performance of the method. Performance, in general, presently exceeds the original multi-laboratory performance data for the listed elements (and others) that are provided in Sec. 13.0. Instrument detection limits (IDLs), lower limits of quantitation (LLOQs) and linear ranges will vary with the matrices, instrumentation, and operating conditions. In relatively simple matrices, IDLs will generally be < 0.1 µg/L. For less sensitive elements (e.g., Se and As) and desensitized major elements, IDLs may be ≥ 1.0 µg/L.

1.3 If Method 6020 is used to determine any analyte not listed in Sec. 1.2, it is the responsibility of the analyst to demonstrate the precision and bias of the method for the waste to be analyzed. The analyst must always monitor potential sources of interferences and take appropriate action to ensure data of known quality (see Sec. 9.0). Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest in the same manner as the listed elements and matrices (see Sec. 9.0).

1.4 Use of this method should be restricted to spectroscopists who are knowledgeable in the recognition and correction of spectral, chemical, and physical interferences in ICP-MS analysis.

1.5 An appropriate internal standard is necessary for each analyte determined by ICP-MS. Recommended internal standards are ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{165}Ho, and ^{209}Bi. The lithium internal standard should have an enriched abundance of ^{6}Li, so that

interference from lithium native to the sample is minimized. Other elements may need to be used as internal standards when samples contain significant native amounts of the recommended internal standards as indicated by high bias of internal standard recoveries.

Note: Other potential causes of a high bias should also be considered before a final decision is made that the internal standard high bias is caused by an excessive concentration of the internal standard isotope in the sample.

1.6 Prior to employing this method, analysts are advised to consult the preparatory method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.7 This method is restricted to use by, or under supervision of, properly experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, aqueous and solid samples are solubilized or digested using the appropriate sample preparation methods (see Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary, if the samples are filtered and acid-preserved prior to analysis (e.g., Methods 3005, 3010, 3015, 3031, 3050, 3051 and 3052). For oils, greases, or waxes, use the solvent dissolution procedure in method 3040 to prepare the samples.

2.2 This method describes multi-element determinations using ICP-MS in environmental samples. The method measures ions produced by a radio-frequency inductively coupled plasma. Analyte species in liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge (m/z) ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or the data flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

3.0 DEFINITIONS

Refer to Chapter One, Chapter Three, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal *m/z* ratio. A data system must be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal.

4.2 Isobaric molecular and doubly charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences that could affect ICP-MS determinations have been identified in the literature (Refs. 3 and 4). Examples include $^{75}ArCl^{+}$ ion on the ^{75}As signal and MoO^{+} ions on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotope abundances from the literature (Ref. 5), the most precise coefficients for an instrument can be determined from the ratio of the net isotope signals *observed* for a standard solution of the interfering element at a concentration which produces sufficient interference at the isotopes of interest that a reliable measurement can be made. Because the ^{35}Cl natural abundance of 75.77% is 3.13 times the ^{37}Cl abundance of 24.23%, the chloride correction for arsenic can be calculated (approximately) as follows (where the $^{38}Ar^{37}Cl^{+}$ contribution at *m/z* 75 is a negligible 0.06% of the $^{40}Ar^{35}Cl^{+}$ signal):

Corrected arsenic signal (using the abundances of natural isotopes for coefficient approximations) =

(*m/z* 75 signal) - (3.13) [(*m/z* 77 signal) - (0.87) (*m/z* 82 signal)]

where, the final term adjusts for any selenium contribution at 77 *m/z*,

NOTE: Arsenic values can be biased high by this type of equation when the net signal at *m/z* 82 is caused by ions other than $^{82}Se^{+}$, (e.g., $^{81}BrH^{+}$ from bromine wastes [Ref. 6]).

NOTE: The coefficients should be verified experimentally using the procedures or coefficients provided by the instrument manufacturer.

Similarly,

Corrected cadmium signal (using the abundances of natural isotopes for coefficient approximations) =

(*m/z* 114 signal) - (0.027)(*m/z* 118 signal) - (1.63)(*m/z* 108 signal)

where, the last 2 terms adjust for any $^{114}Sn^+$ or $^{114}MoO^+$ contributions at *m/z* 114.

NOTE: Cadmium values will be biased low by this type of equation when $^{92}ZrO^+$ ions contribute at *m/z* 108, but use of *m/z* 111 for Cd is even subject to direct ($^{94}ZrOH^+$) and indirect ($^{90}ZrO^+$) additive interferences when Zr is present.

NOTE: With respect to the arsenic equation above, the coefficients could be improved. For example, the coefficient to modify “3.13” (in the equation above) for a particular instrument can be determined from the observed ratio of the m/z 75 to the m/z 77 net isotope signals for a solution of hydrochloric acid. The concentration of HCl used should provide enough signal at the measured isotopes to ensure that a reliable measurement can be made, while not exceeding the linear range of the detector.

The accuracy of these types of equations is based upon the constancy of the *observed* isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found (Ref. 7) to be reliable, e.g., oxide levels can vary with operating conditions. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferent. For example, this type of correction has been reported (Ref. 7) for oxide-ion corrections using ThO^+/Th^+ for the determination of rare earth elements. The use of aerosol desolvation and/or mixed gas plasmas have been shown to greatly reduce molecular interferences (Ref. 8). These techniques can be used, provided that IDL, bias, and precision specifications for analysis of the samples can be met.

4.3 As technology continues to develop, modifications to existing ICP-MS instrumentation can reduce or completely remove common interferences thus eliminating the need for reliance on correction equations. Instruments must be able to demonstrate successful freedom from interferences. Examples of such modifications are discussed in more detail below:

4.3.1 Recent ICP-MS instruments may include collision or reaction cells for removal of molecular isobaric interferences. This type of interference removal is effective, and highly recommended for complex and/or varying matrices. The systems work either by collision of molecular species with an inert gas (usually helium) or by reaction of molecular species or the target analyte with reactive gases (e.g., ammonia or methane). Manufacturer recommendations should be followed for the configuration of the collision/reaction cell. This technique may eliminate the need for most correction equations, but freedom from interference still needs to be demonstrated using the spectral interference check (SIC) solutions described in sections 7.23 and 9.9.

4.3.2 High resolution ICP-MS instruments are available based on several mass analyzer designs with much higher mass resolution within the mass range of traditional ICP-MS instruments. These mass analyzers are not based on quadrupole mass analyzers and have orders of magnitude resolution above quadrupoles, which helps reduce or eliminate interference from polyatomic ions with the same nominal mass. These mass analyzers reduce or eliminate the need for most correction equations, but the instrument needs to be operated at sufficient resolution to remove the expected

interference. For example, resolving ^{52}Cr from $^{40}Ar^{12}C$ requires a resolution of around 4000, while resolving ^{75}As from $^{40}Ar^{35}Cl$ requires a resolution of around 8000. Freedom from interferences needs to be demonstrated for the particular higher resolution mass analyzers ICP-MS.

4.4 Additionally, solid-phase chelation may be used to eliminate isobaric interferences from both element and molecular sources. An on-line method has been demonstrated for environmental waters such as sea water, drinking water and acid decomposed samples. Acid decomposed samples refer to samples decomposed by methods similar to methods 3052, 3051, 3050 or 3015. Samples with% levels of iron and aluminum should be avoided. The method also provides a method for preconcentration to enhance detection limits simultaneously with elimination of isobaric interferences. The method relies on chelating resins such as imminodiacetate or other appropriate resins and selectively concentrates the elements of interest while eliminating interfering elements from the sample matrix. By eliminating the elements that are direct isobaric interferences or those that form isobaric interfering molecular masses, the mass region is simplified and these interferences cannot occur. The method has been proven effective for the certification of reference materials and validated using reference materials (Refs. 13-15). The method has the potential to be used on-line or off-line as an effective sample preparation method specifically designed to address interference problems.

4.5 Since commercial quadrupole ICP-MS instruments nominally provide unit resolution at 10% of the peak height, very high ion currents at adjacent masses can also contribute to ion signals at the mass of interest. Although this type of interference is uncommon, it is not easily corrected, and samples exhibiting a significant problem of this type could need resolution improvement, matrix separation, or analysis using another verified and documented isotope, or otherwise the use of another method.

4.6 Physical interferences are associated with the sample nebulization and transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement (Ref. 9). Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Dissolved solid levels below 0.2% (2,000 mg/L) have been currently recommended (Ref. 10) to minimize solid deposition, although currently-available ICP-MS systems may be able to tolerate much higher levels. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes (Ref. 11). When intolerable physical interferences are present in a sample, a significant suppression of the internal standard signals (to less than 30% of the signals in the calibrations standard) will be observed. Dilution of the sample five-fold (i.e., dilute one part sample with four parts diluent [1:5 = 1+4]) will usually eliminate the problem.

4.7 Memory interferences or carry-over can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

4.8 Reagents and sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents may be necessary. Refer to each method to be used for specific guidance on QC procedures.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a hood and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

5.3 **Hydrofluoric acid is a very toxic acid and penetrates the skin and tissues deeply if not treated immediately.** Injury occurs in two stages: firstly, by hydration that induces tissue necrosis; and secondly, by penetration of fluoride ions deep into the tissue and thereby reacting with calcium. Boric acid and/or other complexing reagents and appropriate treatment agents should be administered immediately.

WARNING: Consult appropriate safety literature for determining the proper protective eyewear, clothing and gloves to use when handling hydrofluoric acid. **Always have appropriate treatment materials readily available prior to working with this acid.** See Method 3052 for additional recommendations for handling hydrofluoric acid from a safety and an instrument standpoint.

5.4 Many metal salts, are extremely toxic if inhaled or swallowed.

WARNING: Exercise extreme care to ensure that samples and standards are handled safely and properly and that all exhaust gases are properly vented. Wash hands thoroughly after handling.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma-mass spectrometer:

6.1.1 The system must be capable of providing resolution, better than or equal to 1.0 u (unified atomic mass unit) at 10% peak height. The system must have a mass range from at least 6 to 240 u and a data system that allows corrections for isobaric interferences and the application of the internal standard technique. Use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution are recommended.

6.1.2 Argon gas, high-purity grade (99.99%).

6.2 Volumetric flasks of suitable material composition, precision and accuracy

6.3 Volumetric pipets of suitable material composition, precision and accuracy

This section does not list all common laboratory ware (e.g., beakers) that might be used.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade, and whenever necessary, ultra-high purity-grade chemicals, must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Reagent water - Reagent water must be interference free. All references to water in this method refer to reagent water unless otherwise specified.

7.3 Ultra high-purity or equivalent acids must be used in the preparation of standards and for sample processing. Redistilled acids are recommended because of the high sensitivity of ICP-MS. Nitric acid at less than 2% (v/v) is necessary for ICP-MS to minimize damage to the interface and to minimize isobaric molecular-ion interferences with the analytes. Many more molecular-ion interferences are observed when hydrochloric and sulfuric acids are used (Refs. 3 and 4). The use of 1% (v/v) HCl is necessary for the stability of antimony and silver concentrations in the range of 50 - 500 µg/L. For concentrations greater than 500 µg/L silver, additional HCl will be needed. As a consequence, the accuracy of analytes that need significant chloride molecular-ion corrections (e.g., As and V) will degrade.

7.3.1 Nitric acid (concentrated), HNO_3

7.3.2 Nitric acid (50% [v/v]), HNO_3 - Prepare by adding 500 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.3.3 Nitric acid (1% [v/v]), HNO_3 - Prepare by adding 10 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.3.4 Hydrochloric acid (concentrated), HCl

7.3.5 Hydrochloric acid (37%), HCl - Prepare by adding 370 mL concentrated HCl to 400 mL water and diluting to 1L.

7.3.6 Hydrofluoric acid (concentrated), HF

7.3.7 Phosphoric acid (concentrated), H_3PO_4

7.3.8 Phosphoric acid (85% [v/v]), H_3PO_4 - Prepare by adding 850 mL concentrated H_3PO_4 to 100 mL water and diluting to 1 L.

7.3.9 Sulfuric acid (concentrated), H_2SO_4

7.3.10 Sulfuric acid (96% [v/v]) H_2SO_4, - Prepare by adding 40 mL water to a 2 L glass beaker. While gently stirring, carefully add 960 mL concentrated H_2SO_4 to the beaker. Mix until combined. Allow to cool. Carefully, quantitatively transfer solution to a 1-L volumetric flask. Bring to volume with additional water if necessary. Mix thoroughly through inversion to combine.

WARNING: Considerable heat is generated upon combining sulfuric acid and water. The use of appropriate personal protection (e.g. proper gloves, safety glasses and protective clothing) is necessary to avoid personal injury such as thermal burns or acid burns due to solution splatter. Also, always add acid to water (rather than water to acid) to reduce splatter.

7.3.11 Citric acid, $HO_2CCH_2C(OH)(CO_2H)CH_2CO_2H$

7.4 Bismuth(III) oxide, Bi_2O_3

7.5 Holmium(III) carbonate pentahydrate, $Ho_2(CO_3)_3•5H_2O$

7.6 Indium (powder), In

7.7 Lithium[^{6}Li] carbonate (95 atom % ^{6}Li), $^6Li_2CO_3$

7.8 Ammonium hexachlororhodate(III), $(NH_4)_3RhCl_6$

7.9 Scandium(III) oxide, Sc_2O_3

7.10 Terbium(III) carbonate pentahydrate, $Tb_2(CO_3)_3•5H_2O$

7.11 Yttrium(III) carbonate, $Y_2(CO_3)_3•3H_2O$

7.12 Ammonium hexafluorotitanate(IV), $(NH_4)_2TiF_6$

7.13 Ammonium molybdate(VI) $(NH_4)_2MoO_4$

7.14 Aluminum(III) nitrate nonahydrate, $Al(NO_3)_3•9H_2O$

7.15 Calcium carbonate, $CaCO_3$

7.16 Iron powder, Fe

7.17 Magnesium oxide, MgO

7.18 Sodium carbonate, Na_2CO_3

7.19 Potassium carbonate, K_2CO_3

7.20 Standard stock solutions - Purchase standard stock solutions from an appropriate commercial source. Otherwise, prepare them manually in the laboratory using only ultra, high-purity grade chemicals or metals (≥ 99.99% purity). See Method 6010 for instructions on preparing standard solutions from solids. Replace stock standards when succeeding dilutions for the preparation of calibration standards cannot be verified.

7.20.1 Bismuth internal standard stock solution (100 µg/mL Bi) - Dissolve 0.1115 g Bi_2O_3 in a minimum amount of dilute HNO_3. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.2 Holmium internal standard stock solution (100 µg/mL Ho) - Dissolve 0.1757 g $Ho_2(CO_3)_3{\bullet}5H_2O$ in 10 mL reagent water and 10 mL concentrated HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.3 Indium internal standard stock solution (100 µg/mL In) - Dissolve 0.1000 g indium in 10 mL concentrated HNO_3. Dilute to 1 L with reagent water.

7.20.4 Lithium internal standard stock solution (100 µg/mL ^6Li) - Dissolve 0.6312 g $^6Li_2CO_3$ (95% atomic abundance) in 10 mL of reagent water and 10 mL concentrated HNO_3. After dissolution is complete, warm the solution to degas. Add 10 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.5 Rhodium internal standard stock solution (100 µg/mL Rh) - Dissolve 0.3593 g $(NH_4)_3RhCl_6$ in 10 mL reagent water. Add 100 mL concentrated HCl and dilute to 1 L with reagent water.

7.20.6 Scandium internal standard stock solution (100 µg/mL Sc) - Dissolve 0.15343 g Sc_2O_3 in 10 mL 50% hot HNO_3. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.7 Terbium internal standard stock solution (100 µg/mL Tb) - Dissolve 0.1828 g $Tb_2(CO_3)_3{\bullet}5H_2O$ in 10 mL 50% HNO_3. After dissolution is complete, warm the solution to degas. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.8 Yttrium internal standard stock solution (100 µg/mL Y) - Dissolve 0.2316 g $Y_2(CO_3)_3{\bullet}3H_2O$ in 10 mL 50% HNO_3. Add 5 mL concentrated HNO_3 and dilute to 1 L with reagent water.

7.20.9 Titanium interference stock solution (100 µg/mL Ti) - Dissolve 0.4133 g $(NH_4)_2TiF_6$ in reagent water. Add 2 drops concentrated HF and dilute to 1 L with reagent water.

7.20.10 Molybdenum interference stock solution (100 µg/mL Mo) - Dissolve 0.2043 g $(NH_4)_2MoO_4$ in reagent water. Dilute to 1 L with reagent water.

7.20.11 Gold preservative stock solution for mercury (100 µg/mL Au) - Purchase as a commercially prepared, high-purity solution of $AuCl_3$ in dilute HCl matrix.

7.21 Mixed-calibration standard solutions - Prepare by diluting stock standard solutions to levels in the linear range for the instrument, using the same combination and concentrations of acids used in the preparation of the sample digestates (approximately 1% HNO_3). The calibration standard solutions must contain a suitable concentration of an appropriate internal standard for each analyte. Internal standards may be added on-line at the time of analysis using a second channel of the peristaltic pump and an appropriate mixing manifold. Generally, an internal standard should be no more than 50 u removed from the analyte. Recommended internal standards include ^{6}Li, ^{45}Sc, ^{89}Y, ^{103}Rh, ^{115}In, ^{159}Tb, ^{169}Ho, and ^{209}Bi. Prior to preparing the mixed standards, each stock standard solution must be analyzed separately to determine possible spectral interferences or the presence of impurities.

NOTE: Care should be taken when preparing the calibration standards to ensure that the elements are compatible and stable when mixed together. Standards which interfere with another analyte, or which are contaminated with another analyte, may not be included in the same calibration standard as that analyte.

Transfer the mixed-standard solutions to an appropriate container for storage. Freshly mixed standards must be prepared as needed with the realization that concentrations can change upon aging. Calibration standards must be initially verified using a QC standard (see Sec. 7.24).

7.22 Blanks - Three types of blanks are necessary for analysis: (1) the calibration blank, which is used in establishing the calibration curve; (2) the method blank, which is used to monitor for possible contamination resulting from the sample preparation procedure; and (3) the rinse blank, which is used to flush the system between all samples and standards.

7.22.1 Calibration blank - Prepare by acidifying reagent water using the same combination and concentrations of acids used in the preparation of the matrix-matched calibration standards (Sec. 7.21) along with the selected concentrations of internal standards, such that there is an appropriate internal standard element for each of the target analytes. The use of HCl for antimony and silver is discussed in Sec. 7.3. The calibration blank will also be used for all initial calibration blank (ICB) and continuing calibration blank (CCB) determinations.

7.22.2 Method blank — Prepare by a processing either a volume of reagent water equal to that used for actual aqueous samples, or, otherwise, a clean, empty container, equivalent to that used for actual solid samples through all of the preparatory and instrument determination steps used for making ICP-MS determinations in samples. These steps may include, but are not limited to, pre-filtering, digestion, dilution, filtering, and analysis (refer to Sec. 9.5).

7.22.3 Rinse blank - Prepare as a 1 - 2% HNO_3 solution. Prepare a sufficient quantity such that it may be used to flush the system in between standards and samples. If mercury is to be analyzed, the rinse blank should also contain 2 µg/mL $AuCl_3$.

7.23 Spectral interference check (SIC) solutions - Prepare so as to contain known concentrations of interfering elements that will demonstrate the appropriate magnitude of interferences and provide an adequate test of any corrections. Chloride in the SIC solution provides a means to evaluate software corrections for chloride-related interferences such as $^{35}Cl^{16}O^+$ on $^{51}V^+$ and $^{40}Ar^{35}Cl^+$ on $^{75}As^+$. Iron is used to demonstrate adequate resolution of the spectrometer for the determination of manganese. Molybdenum serves to indicate oxide effects on cadmium isotopes. The other components are present to evaluate the ability of the measurement system to correct for various molecular-ion isobaric interferences. The SIC is used to verify that the interference levels are corrected by the data system within appropriate QC limits.

NOTE: The final SIC solution concentrations in Table 1 are intended to evaluate corrections for known interferences on only the analytes identified in Sec. 1.0. If the test method is to be used to determine other element(s), it is the responsibility of the analyst to modify the SIC solution accordingly, or prepare an alternative SIC solution, so as to allow adequate verification of interference corrections on the additional element(s) (see Sec. 9.9).

7.23.1 Mixed stock SIC solutions - Prepare the SIC stock solutions using only ultra-pure reagents. They can be obtained commercially or prepared using the following procedures:

7.23.1.1 Mixed SIC stock solution I - Prepare by adding 13.903 g $Al(NO_3)_3 \cdot 9H_2O$, 2.498 g $CaCO_3$ (previously dried at 180 EC for 1 hr), 1.000 g Fe, 1.658 g MgO, 2.305 g Na_2CO_3 and 1.767 g K_2CO_3 to 25 mL of reagent water. Slowly add 40 mL of (50%) HNO_3. After dissolution is complete, warm the solution to degas. Cool and dilute to 1 L with reagent water.

7.23.1.2 Mixed SIC stock solution II - Prepare by slowly adding 7.444 g 85% H_3PO_4, 6.373 g 96% H_2SO_4, 40.024 g 37% HCl, and 10.664 g citric acid ($C_6O_7H_8$) to 100 mL of reagent water. Dilute to 1 L with reagent water.

7.23.2 Mixed working SIC solution - Prepare by combining 10.0 mL of SIC stock solution I, 2.0 mL each of 100-µg/mL titanium stock solution and 100-µg/mL molybdenum stock solution, and 5.0 mL of SIC stock solution II. Dilute to 100 mL with reagent water. Prepare fresh weekly.

7.24 Initial calibration verification (ICV) standard - Prepare by combining compatible metals from standard stock solution sources that differ from those used for the preparation of the calibration standards. The ICV should be prepared so as to contain metal concentrations that are near, but not equal to, the midpoint concentration level of the calibration curve.

7.25 Continuing calibration verification (CCV) standard - Prepare using the same acid matrix and stock standards employed when preparing the calibration standards. The CCV should be prepared so as to contain metal concentrations equal or nearly equivalent to the midpoint concentration of the calibration curve.

7.26 Mass spectrometer tuning solution - Prepare so as to contain elements that represent all of the mass regions of interest (i.e., 10 µg/L Li, Co, In, and Tl) in order to verify that

the resolution and mass calibration of the instrument are within the designated specifications (see Sec. 10.1).

7.27 If the determination of one or more metals using a non-aqueous solvent is required, then all standards and quality control samples must be prepared on a weight/weight basis in the non-aqueous solvent since the density of non-aqueous solvents is not uniform. Standards and quality control materials containing organometallic materials that are soluble in non-aqueous solvents are available from a variety of vendors.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

See Chapter Three, Inorganic Analytes, for sample collection and preservation instructions.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over those criteria given in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800 for QC procedures to ensure the proper operation of the various sample preparation techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800.

9.3 Instrument Detection Limits

Instrument detection limits (IDLs) are useful means to evaluate the instrument noise level and response changes over time for each analyte from a series of reagent blank analyses to obtain a calculated concentration. They are not to be confused with the lower limit of quantitation, nor should they be used in establishing this limit. It may be helpful to compare the calculated IDLs to the established lower limit of quantitation, however, it should be understood that the lower limit of quantitation needs to be verified according to the guidance in Sec. 9.8. IDLs in µg/L can be estimated as the mean of the blank result plus three times the standard

deviation of 10 replicate analyses of the reagent blank solution. (Use zero for the mean if the mean is negative). Each measurement should be performed as though it were a separate analytical sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDLs should be determined at least once using new equipment, after major instrument maintenance such as changing the detector, and/or at a frequency designated by the project. An instrument log book should be kept with the dates and information pertaining to each IDL performed.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination by generating data of acceptable precision and bias for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. It is recommended that the laboratory should repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment that come into direct contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are digested and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If an interference is observed that would prevent the determination of the target analyte, determine the source and eliminate it, if possible, before processing the samples. The method blank should be carried through all stages of sample preparation and instrument determination procedures. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Linear range

The linear range establishes the highest concentration that may be reported without diluting the sample. Following calibration, the laboratory may choose to analyze a standard at a higher concentration than the high standard in the calibration. The standard must recover within 10% of the true value, and if successful, establishes the linear range. The linear range standards must be analyzed in the same instrument run as the calibration they are associated with (i.e., on a daily basis) but may be analyzed anywhere within that run. If a linear range standard is not analyzed for any specific element, the highest standard in the calibration becomes the linear range.

9.7 Sample QC for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, and sensitivity). At a minimum, this should include the

analysis of QC samples including a method blank, a matrix spike (MS), a laboratory control sample (LCS), and a duplicate sample in each analytical batch. Any method blanks, LCS, MS samples, and duplicate samples should be subjected to the same preparatory and instrument determination procedures as those used on actual samples (see Sec. 11.0).

9.7.1 For each batch of samples analyzed, at least one method blank must be carried throughout the entire sample preparation and instrument determination process, as described in Chapter One. The importance of the method blank is to aid in identifying when and/or if sample contamination is occurring. The method blank is considered to be acceptable if it does not contain the target analytes at concentration levels that exceed the acceptance limits defined in Chapter One or in the project-specific DQOs. The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is not reliable because it is based on a single method blank value rather than a statistically determined blank concentration.

Blanks are generally considered to be acceptable if target analyte concentrations are less than ½ the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e. targets are not present in samples or sample concentrations **are ≥10X** the blank). Other criteria may be used depending on the needs of the project.

If the method blank fails to meet the necessary acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples associated with the method blank must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples. If the method blank results do not meet the acceptance criteria and reanalysis is not practical, then the laboratory should report the sample results along with the method blank results, and provide a discussion of the potential impact of the contamination on the sample results. However, if an analyte of interest is found in a sample in the batch near its concentration confirmed in the blank, the presence and/or concentration of that analyte should be considered suspect and may require qualification. Refer to Chapter One for additional guidance regarding the proper protocol when analyzing method blanks.

9.7.2 Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair for each batch of samples processed, at a minimum frequency of one per every 20 samples, as described in Chapter One. An MS/MSD pair is used to document the bias and precision of a method in a given sample matrix. The decision on whether to prepare and analyze duplicate samples or an MS/MSD pair must be based on knowledge of the samples in the analysis batch. If samples are expected to contain target analytes above the LLOQ, laboratories may choose to use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes above the LLOQ, the laboratories should use an MS/MSD pair.

MS/MSD samples should be spiked with each target element at the project-specific action levels, or, when lacking project-specific action levels, between the low- and mid-level standards, as appropriate. Acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses, for each matrix type being analyzed. However, historically derived acceptance limits must not exceed ± 25%

recovery of the target element spike values for bias, and ≤ 20 relative percent difference (RPD) for precision. In the absence of historical data, MS/MSD acceptance limits should be set at ± 25% recovery and ≤ 20 RPD. Refer to Sec. 4.0 of Chapter One for further guidance. If the bias and precision indicators in an analytical batch fail to meet the acceptance criteria, then the interference test discussed in Sec. 9.10 should be performed. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols.

NOTE: If the background sample concentration is very low or non-detect, a spike of greater than 5 times the background concentration is still acceptable. To assess data precision with duplicate analyses, it is preferable to use a high concentration field sample to prepare unspiked laboratory duplicates for metals analyses.

Calculate the RPD between duplicate or MS determinations as follows:

$$RPD = \frac{|D_1 - D_2|}{\left(\frac{|D_1 + D_2|}{2}\right)} \times 100$$

where:

RPD = relative percent difference
D_1 = MS or first sample analysis value
D_2 = MSD or duplicate sample analysis value

9.7.3 At least one LCS should be prepared and analyzed with each batch of analytical samples processed, at a minimum frequency of one LCS per every 20 samples, as described in Chapter One. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS should be spiked at the same levels and using the same spiking materials as the corresponding MS/MSD (see above Sec. 9.7.2). When the results of the MS analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can acceptably perform the analysis in a clean matrix.

LCS acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses. However, historically derived acceptance limits must not exceed ± 20% of the target element spike values. In the absence of historical data, LCS acceptance limits should be set at ± 20%. If the result of an LCS does not meet the established acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples associated with the LCS must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples.

9.7.4 Reference materials containing known amounts of target elements are recommended when an appropriately similar medium of interest are available as one type of QC after appropriate sample preparation. The reference material may be used as the LCS. For soil reference materials, the manufacturers' established acceptance criterion should be used. For solid reference materials, ± 20% (see Sec. 9.7.3) recovery of the reported manufacturers' target element values may not be achievable. Refer to Chapters One and Three for additional information.

9.8 Lower Limit of Quantitation (LLOQ) check standard

9.8.1 The laboratory should establish the LLOQ as the lowest point of quantitation which, in most cases, is the lowest concentration in the calibration curve. The LLOQ is initially verified by the analysis of at least 7 replicate samples, spiked at the LLOQ and processed through all preparation and analysis steps of the method. The mean recovery and relative standard deviation of these samples provide an initial statement of precision and accuracy at the LLOQ. In most cases the mean recovery should be +/- 35% of the true value and RSD should be ≤ 20%. In-house limits may be calculated when sufficient data points exist. Monitoring recovery of LLOQ over time is useful for assessing precision and bias. Refer to a scientifically valid and published method such as Chapter 9 of Quality Assurance of Chemical Measurements (Taylor 1987) or the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs (http://water.epa.gov/scitech/methods/cwa/det/index.cfm) for calculating precision and bias for LLOQ.

9.8.2 Ongoing LLOQ verification, at a minimum, is on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with clean control material (e.g., reagent water, method blanks, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix (free of target compounds). Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated project-specific requirements.

9.9 Verify the magnitude of elemental and molecular-ion isobaric interferences and the adequacy of any corrections at the beginning of an analytical run or once every 12 hours of continuing sample analysis, whichever is more frequent. Do this by analyzing the SIC solution. Results for the unspiked elements in the SIC solution should be less than 2 times the LLOQ. Note that it may not be possible to obtain SIC spiking solutions that are completely free of the unspiked elements. If the presence and concentration of an unspiked element can be confirmed via vendor documentation and/or determination of multiple isotopes of the element in the correct ratios, the concentration actually present may be subtracted from the determined value prior to comparing to the LLOQ limits. Refer to Sec. 4.0 for a discussion on interferences and potential solutions to those interferences if additional guidance is needed.

9.10 The intensities of each internal standard must be monitored for every analysis to ensure that it does not decrease below 30%, with respect to its intensity during the initial calibration. If this occurs, a significant matrix effect must be suspected. Under these conditions, the IDL has degraded, and therefore the correction capability of the internal-standardization technique must then be questioned. If this happens, perform the following procedure:

9.10.1 Make sure the instrument has not drifted by observing the internal standard intensities in the nearest clean matrix, i.e., the calibration blank. If the low internal standard intensities are also observed in the nearby calibration blank, terminate the analysis, correct the problem, recalibrate the instrument, verify the new calibration, and reanalyze the affected samples.

9.10.2 If drift has not been demonstrated to occur as outlined in Sec. 9.10.1, matrix effects need to be removed by diluting the affected sample. Dilute the sample five-

fold (1:5), taking into consideration the need to add the appropriate amounts of internal standards, and reanalyze. If the first dilution does not eliminate the problem, repeat the dilution procedure in an iterative fashion, using ever-increasing dilutions, until the internal-standard intensities exceed the 30% acceptance limit. Correct the reported results using the appropriate dilution factors.

9.11 To obtain analyte data of known quality, it is necessary to measure more than the analytes of interest in order to apply corrections or to determine whether interference corrections are necessary. For example, tungsten oxide molecular-ion species can be very difficult to distinguish from mercury isotopes. If the concentrations of interference sources (such as C, Cl, Mo, Zr, W) are such that, at the correction factor, the analyte is less than the LLOQ and the concentration of interferents are insignificant, then the data may go uncorrected.

NOTE: Monitoring the interference sources does not inevitably necessitate monitoring of the interferant itself, but that a molecular species may be monitored to indicate the presence of the interferent.

When correction equations are used, all QC criteria must also be met. Extensive QC for interference corrections is needed at all times. The monitored masses must include those elements whose hydrogen, oxygen, hydroxyl, chlorine, nitrogen, carbon and sulfur molecular ions could impact the analytes of interest. Unsuspected interferences may be detected by adding pure major matrix components to a sample to observe any impact on the analyte signals. When an interference source is present, the sample elements impacted must be flagged to indicate (a) the percentage interference correction applied to the data; or (b) an uncorrected interference, by virtue of the elemental equation used for quantitation. The isotope proportions for an element or molecular-ion cluster provide information useful for QA.

NOTE: Only isobaric elemental, molecular, and doubly charged interference corrections, which employ the observed isotopic-response ratios or parent-to-oxide ratios (provided an oxide internal standard is used as described in Sec. 4.2) for each instrument system, are acceptable corrections for use in this method.

9.12 It is recommended that the laboratory adopt additional QA practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze reference materials and participate in relevant performance evaluation (PE) studies.

9.13 If less than acceptable bias and precision data are generated for the matrix spike(s), the additional QC protocols in Sections 9.13.1 and/or 9.13.2 should be performed prior to reporting concentration data for the elements in this method. At a minimum these tests should be performed with each batch of samples prepared/analyzed with corresponding unacceptable data quality results. If matrix interference effects are confirmed, then an alternative test method should be considered or the current test method modified, so that the analysis is not affected by the same interference. The use of a standard-addition analysis procedure may also be used to compensate for this effect (refer to Method 7000).

9.13.1 Dilution test

If the analyte concentration is within the linear range of the instrument and sufficiently high (minimally, a factor of 25 times greater than the LLOQ), an analysis of a 1:5 dilution should agree to within ± 20% of the original determination. If not, then a chemical or physical interference effect must be suspected. The matrix spike is often a good choice of sample for the dilution test, since reasonable concentrations of most analytes are present. Elements that fail the dilution test are reported as estimated values.

9.13.2 Post-digestion MS

If a high concentration sample is not available for performing the dilution test, then a post-digestion MS should be performed. The test only needs to be performed for the specific elements that failed original matrix spike limits, and only if the spike concentration added was greater than the concentration determined in the unspiked sample. Following preparation, which may include, but is not limited to, pre-filtration, digestion, dilution and filtration, an aliquot, or dilution thereof, should be obtained from the final aqueous, unspiked-analytical sample, and spiked with a known quantity of target elements. The spike addition should be based on the indigenous concentration of each element of interest in the sample. The recovery of the post-digestion MS should fall within a ± 25 % acceptance range, relative to the known true value, or otherwise within the laboratory-derived acceptance limits. If the post-digestion MS recovery fails to meet the acceptance criteria, the sample results must be reported as estimated values.

9.14 Ultra-trace analysis necessitates the use of clean chemistry practices. Several suggestions for the reduction of contaminants in the analytical blank are provided in Chapter Three, Inorganic Analytes.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Conduct mass calibration and resolution verification checks in the mass regions of interest using the mass spectrometer tuning solution (Sec. 7.26). The mass calibration and resolution verification acceptance criteria must be met prior to the analysis of samples. If the mass calibration differs by more than 0.1 u from the true value, then the mass calibration must be adjusted to the correct value. The resolution must also be verified to be less than 0.9 u full width at 10% peak height.

10.2 At a minimum, the elements required for the project plus any required for interference correction must be calibrated. Recommended isotopes for the analytes in Sec. 1.2 are provided in Table 2. Flush the system in between each standard and sample using the rinse blank (Sec. 7.22.3). The rinse time needs to be sufficient to ensure that analytes present in the linear range are effectively cleaned out prior to analysis of the subsequent sample. Use the average of at least three readings (of a single injection) for both calibration standard and sample analyses.

10.3 Calibration standards should be prepared on an as-needed basis unless stability warrants preparing fresh daily, (or each time a batch of samples is analyzed). If the ICV standard is prepared daily and the results of the ICV analyses meet the acceptance criteria,

then the calibration standards do not need to be prepared daily and may be prepared and stored for as long as the calibration standard viability can be verified through the use of the ICV. If the ICV fails to meet the acceptance criteria, trouble shoot the situation, and then prepare a new set of calibration standards if needed and recalibrate the instrument

10.4 A calibration curve must be analyzed daily. The instrument may be calibrated using a single point standard and a calibration blank (ICB) or a multipoint calibration curve. If a multipoint curve is used a minimum of three standards are required and the correlation coefficient (r) should be $\geq$ 0.995 or the coefficient of determination (r^2) should be $\geq$ 0.990. Relative Standard Error may be used as an alternative to r or r^2, and should be $\leq$ 20%. If a multipoint calibration is used the low standard must be at or below the LLOQ.

NOTE: Inversely weighted linear regressions or other methods may be used in order to minimize curve fitting errors at the low end of the calibration curve.

10.5 After the initial calibration is completed it is verified using several checks.

10.5.1 Initial Calibration Verification (ICV) - The ICV is a standard prepared from a different source than the initial calibration standards. It is analyzed at approximately the mid-level of the calibration and serves as a check that the initial calibration standards are at the correct concentrations. The acceptance range is 90-110% of the true value.

10.5.2 Low-level readback or verification - For a multi-point calibration, the low level standard should quantitate to within 80-120% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at or below the LLOQ is analyzed and should recover within 80-120% of the true value.

10.5.3 Mid-level readback or verification - For a multi-point calibration, the mid-level standard should quantitate to within 90-110% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at the mid-point of the linear range is analyzed and should recover within 90-110% of the true value.

10.5.4 Initial Calibration blank (ICB) - If a multi-level calibration is used, an ICB is analyzed immediately after the calibration (or after the ICV) and must not contain target analytes above half the LLOQ. If a single point calibration is used, the calibration is forced through the ICB, but a second ICB is analyzed as a check and must not contain target analytes above half the LLOQ. If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated.

NOTE: After cleaning the sampler and skimmer cones, improved performance in calibration stability has been observed by method users if the instrument is exposed to the SIC solution. Improved performance has also been observed if the instrument is allowed to rinse for 5 - 10 minutes before starting the calibration process.

10.5.5 Verify the ongoing validity of the calibration curve after every 10 samples, and at the end of each analysis batch run, through the analysis of a CCV standard (Sec. 7.25) and a CCB (Sec. 7.22.1). For the curve to be considered valid the analysis result of the CCV standard must be within ± 10% of its true value and the CCB must not contain target analytes above the LLOQ. If the calibration cannot be verified, sample analysis

must be discontinued, the cause of the problem determined and the instrument recalibrated. All samples following the last acceptable CCV standard must be reanalyzed Flow-injection systems may be used as long as they can meet the performance criteria of the method.

11.0 PROCEDURE

11.1 Preliminary treatment of most samples is necessary because of the complexity and variability of sample matrices. Groundwater samples which have been pre-filtered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix-matched with the standards (i.e., acid concentrations should match). Solubilization and digestion procedures are presented in Chapter Three, Inorganic Analytes.

NOTE: If mercury is to be analyzed, the digestion procedure must use mixed nitric and hydrochloric acids through all steps of the digestion. Mercury will be lost if the sample is digested when hydrochloric acid is not present. If it has not already been added to the sample as a preservative, Au should be added to give a final concentration of 2 mg/L (use 2.0 mL of gold preservative stock (Sec. 7.20.11) per 100 mL of sample) to preserve the mercury and to prevent it from plating out in the sample introduction system.

11.2 Initiate an appropriate operating configuration of the instrument computer according to the instrument manufacturer's instructions.

11.3 Set up the instrument with the proper operating parameters according to the instrument manufacturer's instructions.

11.4 Operating conditions

Tune the instrument by following the instructions provided by the instrument manufacturer. Allow at least 30 minutes for the instrument to equilibrate before analyzing samples.

NOTE: The instrument should have features that protect it from high ion currents. If not, precautions must be taken to protect the detector. A channel electron multiplier or active film multiplier will suffer from fatigue after being exposed to high ion currents. This fatigue can last from several seconds to hours depending on the extent of exposure. During this time period, response factors are constantly changing, which invalidates the calibration curve, causes instability, and invalidates sample analyses.

11.5 Calibrate the instrument following the procedure outlined in Sec. 10.0.

11.6 Flush the system with the rinse blank solution (Sec. 7.22.3) until the signal levels return to the data quality objectives or method LLOQs (usually about 30 seconds) before the analysis of each sample. Nebulize each sample until a steady-state signal is achieved (usually about 30 seconds) prior to collecting data.

11.7 Dilute and reanalyze samples that exceed the linear range for an analyte (or species needed for a correction) or measure an alternate, but less-abundant, isotope. The

linearity at the alternate mass must be confirmed by appropriate calibration (see Sec. 10.4). Alternatively apply solid-phase chelation chromatography to eliminate the matrix as described in Sec. 4.3.

11.8 Determination of percent dry weight

When sample results are to be calculated on a dry-weight basis, a separate portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

CAUTION: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

11.8.1 Immediately after weighing the sample aliquot to be digested, weigh an additional 5- to 10-g aliquot of the sample to the nearest 0.01g into a tared crucible. Dry this aliquot overnight at 105 EC. Allow the sample to cool in a desiccator before weighing.

11.8.2 Calculate the % dry weight as follows:

$$\%\ \text{dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

This oven-dried aliquot is not used for the extraction and should be appropriately disposed of once the dry weight is determined.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 If dilutions were performed, apply the appropriate corrections to the sample values.

12.2 If appropriate, or required by the project or regulation for data reporting, calculate results for solids on a dry-weight basis as follows:

$$Concentration_{DW} = \frac{C \times V}{W \times S}$$

where:

$Concentration_{DW}$ = Concentration on a dry weight basis (mg/kg)
C = Digest concentration (mg/L)
V = Final volume after sample preparation (L)
W =Wet sample mass (kg)
S = % Solids/100 = % dry weight/100

Calculations must include appropriate interference corrections (see Sec. 4.2 for examples), internal-standard normalization, and the summation of signals at 206, 207, and 208 *m/z* for lead (to compensate for any differences in the abundances of these isotopes between samples and standards).

13.0 METHOD PERFORMANCE

Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. <u>These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.</u>

Table 3 summarizes the method performance data for aqueous and sea water samples with interfering elements removed and samples preconcentrated prior to analysis. Table 4 summarizes the performance data for a simulated drinking water standard. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety,
http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the ACS publication listed in Sec. 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The pages to follow contain the tables, and figures referenced by this method.

TABLE 1

RECOMMENDED SPECTRAL INTERFERENCE CHECK (SIC) SOLUTION COMPONENTS AND CONCENTRATIONS

Solution Component	SIC Concentration (mg/L)
Al	100.0
Ca	300.0
Fe	250.0
Mg	100.0
Na	250.0
P	100.0
K	100.0
S	100.0
C	200.0
Cl	2000.0
Mo	2.0
Ti	2.0

TABLE 2

RECOMMENDED ELEMENTAL ISOTOPES FOR SELECTED ELEMENTS

Element of Interest	Mass of Isotope
Aluminum	**<u>27</u>**
Antimony	121, **<u>123</u>**
Arsenic	**<u>75</u>**
Barium	138, 137, 136, **<u>135</u>**, 134
Beryllium	**<u>9</u>**
Bismuth (IS)	209
Cadmium	**<u>114</u>**, 112, **<u>111</u>**, 110, 113, 116, 106
Calcium (I)	42, 43, **<u>44</u>**, 46, 48
Chlorine (I)	35, 37, (77, 82)[a]
Chromium	**<u>52</u>**, **<u>53</u>**, **<u>50</u>**, 54
Cobalt	**<u>59</u>**
Copper	**<u>63</u>**, **<u>65</u>**
Holmium (IS)	165
Indium (IS)	**<u>115</u>**, 113
Iron (I)	**<u>56</u>**, **<u>54</u>**, **<u>57</u>**, 58
Lanthanum (I)	139
Lead	**<u>208</u>**, **<u>207</u>**, **<u>206</u>**, 204
Lithium (IS)	6[b], 7
Magnesium (I)	24, **<u>25</u>**, **<u>26</u>**
Manganese	**<u>55</u>**
Mercury	202, **<u>200</u>**, 199, 201
Molybdenum (I)	98, 95, 96, 92, **<u>97</u>**, 94, (108)[a]
Nickel	58, **<u>60</u>**, 62, **<u>61</u>**, 64
Potassium (I)	**<u>39</u>**
Rhodium (IS)	103
Scandium (IS)	45
Selenium	80, **<u>78</u>**, **<u>82</u>**, **<u>76</u>**, **<u>77</u>**, 74
Silver	**<u>107</u>**, **<u>109</u>**
Sodium (I)	**<u>23</u>**
Terbium (IS)	159
Thallium	**<u>205</u>**, 203
Vanadium	**<u>51</u>**, **<u>50</u>**
Tin (I)	120, **<u>118</u>**
Yttrium (IS)	89
Zinc	64, **<u>66</u>**, **<u>68</u>**, **<u>67</u>**, 70

<u>NOTE</u>: Method 6020 is recommended for only those analytes listed in Sec.1.2. Other elements are included in this table because they are potential interferents (labeled I) in the determination of recommended analytes, or because they are commonly used internal standards (labeled IS). Isotopes are listed in descending order of natural abundance. The most generally useful isotopes are underlined and in boldface, although certain matrices may necessitate the use of alternative isotopes.

[a] These masses are also useful for interference correction (Sec. 4.2).

[b] Internal standard must be enriched in the ^{6}Li isotope. This minimizes interference from indigenous lithium.

TABLE 3

METHOD PERFORMANCE DATA FOR AQUEOUS AND SEA WATER SAMPLES [a]
WITH INTERFERING ELEMENTS REMOVED AND SAMPLES PRECONCENTRATED PRIOR TO ANALYSIS

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL) [b]		
		9.0 mL	27.0 mL	CERTIFIED
Manganese	55	1.8±0.05	1.9±0.2	1.99±0.15
Nickel	58	0.32±0.018	0.32±0.04	0.30±0.04
Cobalt	59	0.033±0.002	0.028±0.003	0.025±0.006
Copper	63	0.68±0.03	0.63±0.03	0.68±0.04
Zinc	64	1.6±0.05	1.8±0.15	1.97±0.12
Copper	65	0.67±0.03	0.6±0.05	0.68±0.04
Zinc	66	1.6±0.06	1.8±0.2	1.97±0.12
Cadmium	112	0.020±0.0015	0.019±0.0018	0.019±0.004
Cadmium	114	0.020±0.0009	0.019±0.002	0.019±0.004
Lead	206	0.013±0.0009	0.019±0.0011	0.019±0.006
Lead	207	0.014±0.0005	0.019±0.004	0.019±0.006
Lead	208	0.014±0.0006	0.019±0.002	0.019±0.006

NOTE: Data obtained from Ref. 12.

[a] The dilution of the sea-water during the adjustment of pH produced 10 mL samples containing 9 mL of sea-water and 30 mL samples containing 27 mL of sea-water. Samples containing 9.0 mL of CASS-2, n=5; samples containing 27.0 mL of CASS-2, n=3.

[b] 95% confidence limits

TABLE 4

ANALYSIS OF NIST SRM 1643b - TRACE METALS IN WATER [a]

ELEMENT	ISOTOPE	CONCENTRATION (ng/mL) [b]	
		DETERMINED	CERTFIED
Manganese	55	30±1.3	28±2
Nickel	58	50±2	49±3
Cobalt	59	27±1.3	26±1
Nickel	60	51±2	49±3
Copper	63	23±1.0	21.9±0.4
Zinc	64	67±1.4	66±2
Copper	65	22±0.9	21.9±0.4
Zinc	66	67±1.8	66±2
Cadmium	111	20±0.5	20±1
Cadmium	112	19.9±0.3	20±1
Cadmium	114	19.8±0.4	20±1
Lead	206	23±0.5	23.7±0.7
Lead	207	23.9±0.4	23.7±0.7
Lead	208	24.2±0.4	23.7±0.7

NOTE: Data obtained from Ref. 12.

[a] 5.0 mL samples, n=5

[b] 95% confidence limits

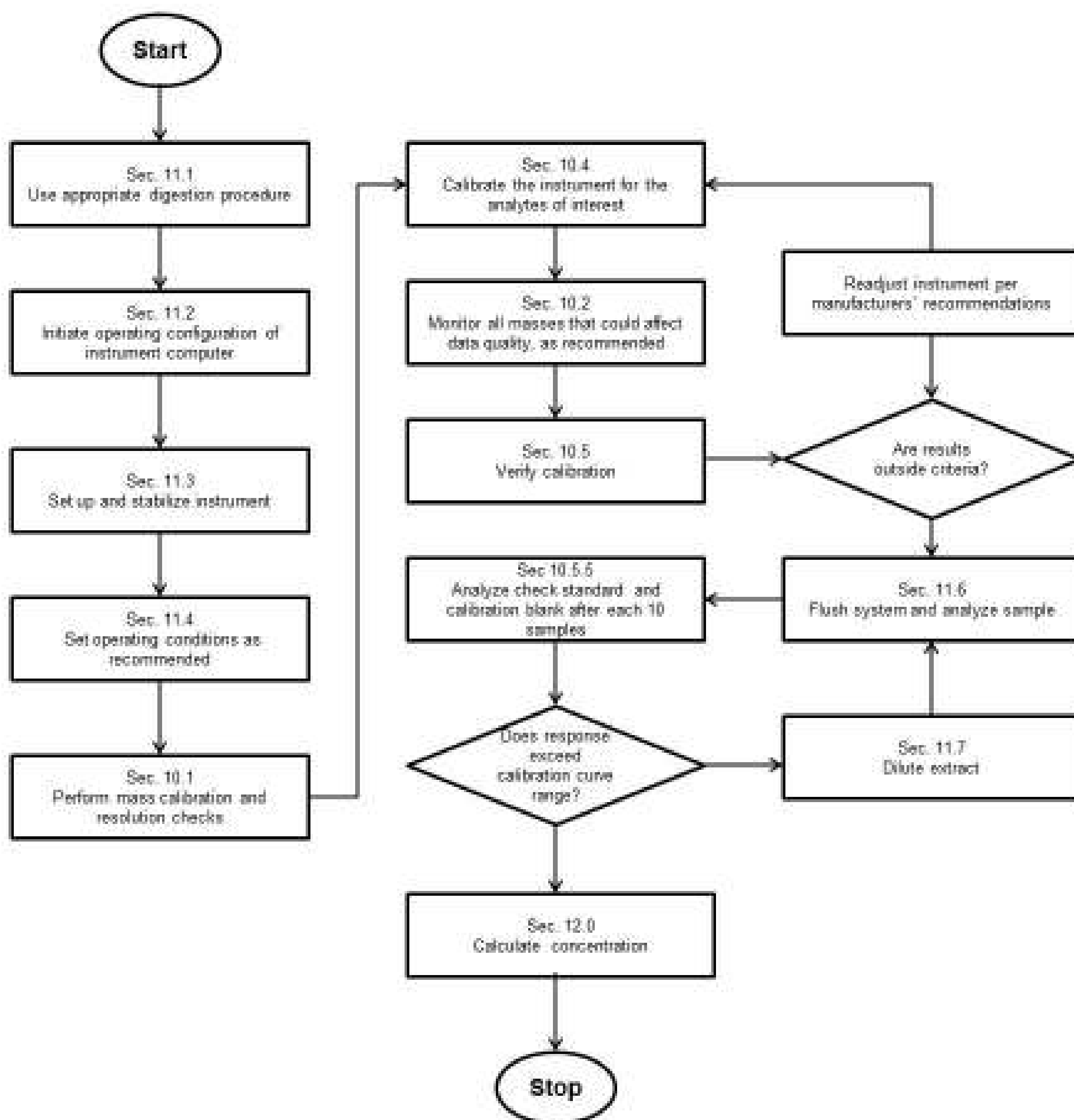
TABLE 5

COMPARISON OF TOTAL MERCURY RESULTS IN HEAVILY CONTAMINATED SOILS

Soil Sample	Mercury in µg/g	
	ICP-MS	CVAA
1	27.8	29.2
2	442	376
3	64.7	58.2
4	339	589
5	281	454
6	23.8	21.4
7	217	183
8	157	129
9	1670	1360
10	73.5	64.8
11	2090	1830
12	96.4	85.8
13	1080	1190
14	294	258
15	3300	2850
16	301	281
17	2130	2020
18	247	226
19	2630	2080

NOTE: Data obtained from Ref. 16.

INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY



Appendix A

Summary of Revisions to Method 6020 (From Revision 1, February 2007):

1. Improved overall method formatting for consistency with new SW-846 methods style guidance.
2. Section 1.2 – Changed "Inductively coupled plasma—atomic emission spectrometry" to "Inductively coupled plasma—optical emission spectrometry".
3. Section 1.6 - inserted references to additional 3000 series preparatory methods to ICP analysis. Also added method 6800 to sections 1.6 and 9.2 as a preparatory method.
4. Inserted additional safety guidance regarding the use of HF.
5. Inserted new section (7.27) regarding analysis of non-aqueous solvents.
6. Reformatted certain paragraphs with the heading "NOTE" or "WARNING" to better denote the importance of the recommendations provided therein.
7. Extensively reformatted "REAGENTS AND STANDARDS" section and to meet current SW-846 method guidelines.
8. Significantly updated and expanded "QUALITY CONTROL" section for better adherence to current SW-846 method guidelines and for improved alignment with current universal practices for published analytical methods.
9. Inserted new sections (Sections 7.23 and 9.9) to describe the preparation and use of the spectral interference check (SIC) solution; also added instructions to match the matrix of this solution to that of the calibration standards.
10. Renamed "QC standard" as "ICV standard" in Sec. 7.24.
11. Added new Sec. 7.25 describing the preparation of a "CCV" standard, consistent with the equivalent section in 6010.
12. Replaced the term "unity" with "uniform" in Section 7.27.
13. Removed all references to method 7000 except for guidance regarding the method of standard addition.
14. The term "accuracy" was replaced by "bias" where appropriate.
15. In Section 9.4, the requirement to repeat the demonstration of proficiency for new staff and instrumentation changes was changed to a recommendation.
16. Section 9.7.2 – Added a note regarding MS/MSD spike concentrations and unspiked laboratory duplicates.
17. The section regarding analysis of reference materials (Sec. 9.7.4) was revised for clarity and the term "Standard Reference Material" was replaced with "reference material" throughout the method.
18. Inserted new section (Sec. 9.8) describing the preparation and use of an LLOQ standard. This section includes two new references for guidance on assessing precision and bias.
19. The section describing matrix interference check samples (Sec. 9.13) has been revised for clarity. The post-digestion MS is only recommended if a high concentration sample is not available for performing the dilution test.
20. Substituted certain terms with new terms (i.e. "must" in place of "shall") to conform with the Performance-based Methods Approach goal of flexibility.
21. Removed reference to "linear dynamic range" as noted by the Inorganic Methods Work Group. Section 9.6 regarding the linear range was added.
22. Mid-level read back or verification standard added to Section 10.5.3.
23. Moved the sentence "If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated." From Section 10.5.5 to Section 10.5.4.
24. Added 95 as mass of isotope for molybdenum.

Appendix B

25. Tables 3 and 4 from 6020A presenting example precision and accuracy data for aqueous and solid matrices were removed.
26. Language was updated in Section 9.7.1 regarding method blanks.

METHOD 6010D

INDUCTIVELY COUPLED PLASMA—OPTICAL EMISSION SPECTROMETRY

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SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data

included in this method are for guidance purposes only and are not intended to be and must not be used as absolute quality control (QC) acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 Inductively coupled plasma—optical emission spectrometry (ICP-OES) is a spectrometric technique used to determine trace elements in aqueous solutions. In ICP-OES, a sample solution is aspirated (i.e., nebulized) continuously into an inductively coupled, argon-plasma discharge, where analytes of interest are converted to excited-state, gas-phase atoms or ions. As the excited-state atoms or ions return to their ground state, they emit energy in the form of light at wavelengths that are characteristic of each specific element. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element in the analyzed sample. Thus, by determining which wavelengths are emitted by a sample and their respective intensities, the elemental composition of the given sample relative to a reference standard may be quantified. For accurate results, direct ICP-OES analysis should be conducted on only relatively clean, aqueous matrices (e.g., pre-filtered groundwater samples). Other, more complex aqueous and/or solid samples need acid digestion prior to analysis; the analyst should ensure that a sample digestion method is chosen that is appropriate for each analyte and the intended use of the data. Refer to Chapter Three for the appropriate digestion procedures.

The following RCRA analytes have been determined by this method:

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Aluminum	Al	7429-90-5	Mercury*	Hg	7439-97-6
Antimony	Sb	7440-36-0	Molybdenum	Mo	7439-98-7
Arsenic	As	7440-38-2	Nickel	Ni	7440-02-0
Barium	Ba	7440-39-3	Phosphorus	P	7723-14-0
Beryllium	Be	7440-41-7	Potassium	K	7440-09-7
Boron	B	7440-42-8	Selenium	Se	7782-49-2
Cadmium	Cd	7440-43-9	Silica	SiO_2	7631-86-9
Calcium	Ca	7440-70-2	Silver	Ag	7440-22-4
Chromium	Cr	7440-47-3	Sodium	Na	7440-23-5
Cobalt	Co	7440-48-4	Strontium	Sr	7440-24-6
Copper	Cu	7440-50-8	Thallium	Tl	7440-28-0
Iron	Fe	7439-89-6	Tin	Sn	7440-31-5
Lead	Pb	7439-92-1	Titanium	Ti	7440-32-6
Lithium	Li	7439-93-2	Vanadium	V	7440-62-2

Element	Symbol	CASRN[a]	Element	Symbol	CASRN[a]
Magnesium	Mg	7439-95-4	Zinc	Zn	7440-66-6
Manganese	Mn	7439-96-5			

[a]Chemical Abstract Service Registry Number

*NOTE: Mercury is not typically analyzed by this method and is not recommended for low-level quantitative analysis; however, this method can be used as a screening tool (e.g., prior to analysis by a low-level method when high concentrations of mercury are expected).

CAUTION: Also note that mercury memory effects may result from the analysis of samples that contain high level Hg concentration. See Method 6020B Sections 7.20.11, 7.22.3, and 11.1 for guidance when analyzing for mercury.

1.2 The table in Section 1.1 lists the elements for which this method has been validated. The sensitivity and the optimum and linear ranges for each element will vary with the wavelength, spectrometer, matrix, and operating conditions. Refer to the manufacturer's instructions for recommended analytical wavelengths and estimated instrument detection limits (IDLs) for the elements in a clean aqueous matrix with insignificant background interferences. Other elements and matrices may be analyzed by this method if acceptable performance at the concentrations of interest is demonstrated (see Sec. 9.0).

1.3 IDLs are necessarily instrument-specific. Therefore, if needed, an IDL must be determined through a separate experimental study for each instrument in a laboratory. IDLs should be established at minimum on an annual basis, for each matrix type analyzed and for each preparatory/determinative method combination used (refer to Chapters One and Three for guidance).

1.4 Analysts should clearly understand the data quality objectives (DQOs) prior to analysis. Before using the method for routine environmental analysis, analysts should document and have on file the necessary initial demonstration of performance (IDP) data, as described in Section 9.0.

1.5 Use of this method is restricted to spectroscopists who are knowledgeable in the correction of the spectral, chemical, and physical interferences described in this method.

1.6 Prior to employing this method, analysts are advised to consult the preparatory method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the DQOs for the intended application.

1.7 This method is restricted to use by, or under supervision of, properly experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Prior to analysis, aqueous and solid samples are solubilized or digested using the appropriate sample preparation methods (see Chapter Three). When analyzing groundwater samples for dissolved constituents, acid digestion is not necessary, if the samples are filtered and acid-preserved prior to analysis (e.g., Methods 3005, 3010, 3015, 3031, 3050, 3051 and 3052). Samples that are not digested necessitate the use of either an internal standard or should be matrix-matched with the standards. If using the former option, the instrument software should be programmed to correct for the intensity differences of the internal standard between samples and standards.

2.2 This method describes multi-element determinations by ICP-OES using sequential or simultaneous optical systems, and axial or radial viewing of the plasma. The ICP-OES instrument measures characteristic emission spectra by optical spectroscopy. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission spectra are produced by a radio-frequency, inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive devices.

2.3 Background correction is necessary for trace element determination. Background emission must be measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement, on either or both sides of the analytical line, will be determined by the complexity of the spectrum adjacent to the analyte line. The position used should be as free as possible from spectral interference and should reflect the same change in background intensity as that which occurs at the analyte wavelength being measured.

Background correction is *not* needed in cases of line broadening, where a background correction measurement would actually degrade the analytical result. Analysts should recognize the possibility of additional interferences, as identified in Sec. 4.0, and make appropriate corrections. Tests for the presence of additional interferences are described in Sec. 9.9. Alternatively, analysts may choose multivariate calibration methods, in which case, point selections for background correction are superfluous, since whole spectral regions are processed.

3.0 DEFINITIONS

Refer to Chapter One, Chapter Three, and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

Interferences can arise from a variety of sources and serve to diminish the bias and precision of analytical data, particularly when determining elements at trace levels. Interferences to ICP-OES have been studied in detail and are well understood. A summary of interferences to ICP-OES analysis as well as techniques to mitigate their effects on data are provided in the sections to follow.

4.1 Spectral interferences can arise from several sources. Techniques to identify and compensate for spectral interferences are discussed below.

4.1.1 Background emission from continuous or recombination phenomena and/or stray light from the line emission of high concentration elements

4.1.1.1 Compensation for background emission and stray light can usually be conducted by subtracting the background emission determined through measurements obtained adjacent to the analyte wavelength peak. Spectral scans of samples or single-element solutions in the analyte regions may indicate when the use of alternate wavelengths is desirable because of severe spectral interference. These scans will also show whether the most appropriate estimate of the background emission is provided by an interpolation from measurements obtained on both sides of the wavelength peak, or by measured emission obtained only on one side.

The locations selected for the measurement of background intensity will be determined by the complexity of the spectrum adjacent to the wavelength peak. The locations used for routine measurement must be free of off-line spectral interference (interelement or molecular), or otherwise adequately corrected to reflect the same change in background intensity as that which occurs at the wavelength peak. For multivariate methods using whole spectral regions, background scans should be included in the correction algorithm. Off-line spectral interferences are handled by including spectra on interfering species in the algorithm.

4.1.1.2 To determine the appropriate location for off-line background correction, the area adjacent to the wavelength on either side must be scanned, so that the apparent emission intensity from all other method analytes may be recorded. This spectral information must be documented and kept on file. The location selected for background correction must be either free of off-line inter-element spectral interference or a computer routine must be used for automatic correction on all determinations. If a wavelength other than the recommended wavelength is used, the analyst must determine and document both the overlapping and nearby spectral interference effects from all method analytes and common elements and provide for their automatic correction on all analyses. Tests to determine spectral interference must be performed using analyte concentrations that will adequately describe the interference. Normally, 100-mg/L, single-element solutions are sufficient. However, for analytes such as iron, that may be found in the sample at high concentration, a more appropriate test would be to use a concentration near the upper limit of the analytical range (refer to Chapter Three for additional guidance).

4.1.2 Overlaps from the molecular spectra of the same target element may be avoided through the use of an alternate wavelength for quantitation.

4.1.3 Optical spectral-line overlaps between target elements

4.1.3.1 Interelement spectral overlaps are typically compensated through the use of equations that correct for interelement contributions. Instruments that use equations for interelement correction necessitate that interfering element(s) are analyzed at the same time as the target element(s) of interest. When operative and uncorrected, interelement interferences will produce false positive or positively biased determinations. However, if the interference affects the point selected for background correction, the resulting overcorrection will cause a negative bias. More extensive information on interferent effects at various wavelengths and resolutions is available in reference wavelength tables and books. Analysts may apply interelement-correction equations determined on their instruments with tested concentration ranges to compensate (off-line or on-line) for the effects of interfering elements. Selected potential spectral interferences observed for the recommended wavelengths are given in Table 1.

4.1.3.2 For multivariate calibration methods that employ whole spectral regions, spectral interferences are handled by including spectra of the interfering elements in the calibration algorithm. The interferences listed in Table 1 are those that occur between method analytes. Only interferences of a direct overlap nature are shown. These overlaps were observed with a single instrument having a working resolution of 0.035 nm.

4.1.3.3 When using interelement-correction equations, the interference may be expressed as analyte concentration equivalents (i.e., false positive analyte concentrations) arising from 100 mg/L of the interference element. For example, if As is to be determined at 193.696 nm in a sample containing approximately 10 mg/L of Al, according to Table 1, 100 mg/L of Al will yield a false positive signal equivalent to an As concentration of approximately 1.3 mg/L. Correspondingly, the presence of 10 mg/L of Al will result in a false positive signal for As equivalent to approximately 0.13 mg/L. The analyst is cautioned that alternate instruments may exhibit somewhat different levels of interference than those shown in Table 1. The interference effects must, therefore, be evaluated for each individual instrument, since the intensities will vary (see Sec. 4.1.3.5). It should also be noted that instruments using an Echelle grating are potentially subject to interferences from different diffraction orders. These potential interferences will not be listed in standard tables of emission lines and therefore careful evaluation of interelement corrections as described in Sec. 9.9.1 becomes even more vital.

4.1.3.4 Interelement corrections will vary for the same emission line among instruments because of differences in resolution. Such differences are determined by the grating, entrance and exit slit widths, and by the order of dispersion. Interelement corrections will also vary depending upon the choice of background correction points. Selecting a background correction point where an interfering emission line may appear should be avoided when practical. Interelement corrections that constitute a major portion of an emission signal may

not yield accurate data. Analysts should continuously note that some samples may contain uncommon elements that could contribute spectral interferences.

4.1.3.5 As already noted (Sec. 4.1.3.3), the interelement effects must be evaluated for each individual instrument, whether configured as a sequential or simultaneous instrument. For each instrument, intensities will vary not only with optical resolution, but also with operating conditions (such as power, viewing height and argon-flow rate). When using the recommended wavelengths, the analyst must determine and document for each wavelength the effect from referenced interferences (Table 1) as well as any other suspected interferences that may be specific to the instrument or matrix. The analyst should utilize a computer routine for automatic correction on all analyses.

4.1.3.6 Analysts of sequential instruments must verify the absence of spectral interference by scanning over a range of 0.5 nm, centered on the wavelength of interest, for several samples. The range for lead, for example, would be from 220.6 - 220.1 nm. The procedure must be repeated whenever a new matrix is to be analyzed and when a new calibration curve using different instrumental conditions is to be prepared. Samples that show an elevated background emission across the range may be background-corrected by applying a correction factor equal to the emission adjacent to the line or at two points on either side of the line and interpolating between them. An alternate wavelength that does not exhibit a background shift or spectral overlap may also be used.

4.1.3.7 The accuracy of interelement corrections should be verified daily through the analysis of spectral-interference check (SIC) solutions. See Secs. 7.12 and 9.9 for instructions on the preparation and use of SIC solutions.

4.1.3.8 When interelement corrections are *not* used, the absence of interferences **must** be verified. Procedures for verifying the absence of interferences are given in sections 7.12 and 9.9.

4.2 Physical interferences are effects associated with the sample nebulization and transport processes. Changes in viscosity and surface tension can cause significant inaccuracies, especially in samples containing high dissolved solids or acid concentrations. If physical interferences are present, they must be reduced through (1) sample dilution, (2) the use of a peristaltic pump; (3) the use of an internal standard; or (4) the use of a high-solids nebulizer.

Another problem that can occur, when high concentrations of dissolved solids are present, is salt buildup at the tip of the nebulizer, thus affecting aerosol flow rate and resulting in instrumental drift. Salt buildup can be controlled through (1) wetting the argon prior to nebulization; (2) use of a tip washer; (3) use of a high-solids nebulizer; or (4) sample dilution. Also, it has been reported that better control of the argon flow rate, especially to the nebulizer, improves instrument performance. This may be accomplished with the use of mass flow controllers. The tests described in Sec. 9.11 will help determine if a physical interference is present.

4.3 Chemical interferences include molecular compound formation, ionization effects, and solute vaporization effects. Normally, these effects are not significant with the ICP technique. However, if observed, they can be minimized by (1) careful selection of operating

conditions (i.e., incident power, observation position, etc.); (2) buffering of the sample through matrix-matching; and (3) standard-addition procedures. Chemical interferences are highly dependent on matrix type and analyte.

4.3.1 The majority of interferences likely to be encountered when using this method can be managed successfully using the techniques discussed throughout Sec. 4.1. However, based on professional judgment, the method of standard additions may be useful when certain specific interferences are encountered. Refer to Method 7000 for a more detailed discussion on the use and application of the method of standard additions.

4.3.2 An alternative to the method of standard additions is the use of an internal standard(s). In the internal standard technique, one or more elements not found in the samples, and verified to not cause an interelement spectral interference, are added to the samples, calibration standards, and blanks. Yttrium or scandium is often used for this purpose. The concentration should be sufficient for optimum precision, but not so high as to alter the salt concentration of the matrix. The internal standard element intensity is used to ratio the analyte intensity signals for both calibration and quantitation. This technique is very useful in overcoming matrix interferences, particularly in high solids matrices.

4.4 Memory interferences result when analytes in a previous sample contribute to the intensity signals measured in a subsequent sample. Memory effects can result from sample deposition on the uptake tubing to the nebulizer and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. The possibility of memory interferences should be considered within an analytical run. When recognized, suitable rinse times should be established to reduce them. The rinse times necessary for a particular element must be estimated prior to analysis. The estimation may be made by aspirating a standard containing the element(s) of interest at a concentration level that is ten times the typical or expected amount, or at the upper limit of the linear range. The aspiration time for the rinse time-estimation standard should be the same as a normal sample analysis period, followed by analysis of the rinse blank at a series of designated intervals. The length of the rinse time necessary for reducing the analyte signal(s) to less than or equal to the IDL should be noted. A rinse period of at least 60 seconds should be used between samples and standards until a more suitable rinse time can be established. If a memory interference is determined to be present, the sample must be reanalyzed following use of the newly established rinse period. Alternate rinse times may be established by the analyst based upon the project-specific DQOs.

4.5 Analysts are advised that high salt concentrations can cause analyte signal suppressions and confuse interference tests. If the instrument does not display negative values, fortify the SIC check solution with the elements of interest at 0.5 - 1 mg/L and measure the added standard concentration accordingly. Concentrations should be within 20% of the true spiked concentration or dilution of the samples will be necessary. In the absence of measurable analyte, overcorrection could go undetected if a negative value is reported as zero.

4.6 The calibration blank (Sec. 7.11.2.1) may restrict the quantitation sensitivity, or otherwise degrade the precision and bias of the analysis. Chapter Three should be consulted for clean chemistry methods and procedures for reducing the magnitude and variability of the calibration blank.

4.7 Reagents and sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents may be necessary. Refer to each method to be used for specific guidance on QC procedures.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDSs) should be available to all personnel involved in these analyses.

5.2 Concentrated nitric and hydrochloric acids are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a hood and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection when working with these reagents.

5.3 **Hydrofluoric acid is a very toxic acid and penetrates the skin and tissues deeply if not treated immediately.** Injury occurs in two stages: firstly, by hydration that induces tissue necrosis; and secondly, by penetration of fluoride ions deep into the tissue and thereby reacting with calcium. Boric acid and/or other complexing reagents and appropriate treatment agents should be administered immediately.

WARNING: Consult appropriate safety literature for determining the proper protective eyewear, clothing and gloves to use when handling hydrofluoric acid. **Always have appropriate treatment materials readily available prior to working with this acid.** See Method 3052 for additional recommendations for handling hydrofluoric acid from a safety and an instrument standpoint.

5.4 Many metal salts, such as those of osmium, are extremely toxic if inhaled or swallowed.

WARNING: Exercise extreme care to ensure that samples and standards are handled safely and properly and that all exhaust gases are properly vented. Wash hands thoroughly after handling.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled, argon-plasma, optical emission spectrometer

6.1.1 Computer-controlled, emission spectrometer with background correction capability

6.1.2 Radio-frequency generator - compliant with FCC regulations

6.1.3 Optional mass-flow controller for argon nebulizer-gas supply

6.1.4 Optional peristaltic pump

6.1.5 Optional autosampler

6.1.6 Argon gas supply - high purity

6.2 Volumetric flasks of suitable material composition, precision and accuracy

6.3 Volumetric pipets of suitable material composition, precision and accuracy

This section does not list all common laboratory ware (e.g., beakers) that might be used.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade, and whenever necessary, ultra-high purity-grade chemicals, must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Reagent water - Reagent water must be interference free. All references to water in this method refer to reagent water unless otherwise specified.

7.3 Hydrochloric acid (concentrated), HCl

7.4 Hydrochloric acid (50% [v/v]), HCl - Prepare by adding 500 mL concentrated HCl to 400 mL water and diluting to 1 L.

7.5 Hydrochloric acid (5% [v/v]), HCl - Prepare by adding 50 mL concentrated HCl to 400 mL water and diluting to 1 L.

7.6 Nitric acid (concentrated), HNO_3

7.7 Nitric acid (50% [v/v]), HNO_3 - Prepare by adding 500 mL concentrated HNO_3 to 400 mL water and diluting to 1 L.

7.8 Hydrofluoric acid (concentrated), HF - For use in matching the background matrices of the calibrations standards relative to those of the samples.

7.9 Standard stock solutions — Purchase standard stock solutions from an appropriate commercial source. Otherwise, prepare them manually in the laboratory using only ultra, high-purity grade chemicals or metals (≥ 99.99% purity). When preparing them manually, **except where specifically noted in the following sections**, dry all metal salts for one hour at 105 °C prior to use. Replace stock standards when succeeding dilutions for the preparation of calibration standards cannot be verified.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

NOTE: This section does not apply when analyzing samples prepared by Method 3040.

NOTE: The mass of each analyte is expressed to four decimal places, since rounding to two decimal places can contribute up to 4% error for some compounds.

7.9.1 Aluminum standard stock solution (1000 µg/mL Al) - Prepare by dissolving exactly 1.000 g of aluminum metal in a beaker containing an acid mixture of 4.0 mL of 50% HCl and 1.0 mL of concentrated HNO_3. Warm the beaker slowly to aid in dissolution of the metal. Afterwards, equilibrate the solution to ambient temperature. Add an additional 10.0 mL of 50% HCl and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.2 Antimony standard stock solution (1000 µg/mL Sb) - Prepare by dissolving exactly 2.6673 g of dried $K(SbO)C_4H_4O_6$ in reagent water. Add 10 mL 50% HCl and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.3 Arsenic standard stock solution (1000 µg/mL As) - Prepare by dissolving exactly 1.3203 g of dried As_2O_3 in 100 mL reagent water containing 0.4 g NaOH. Acidify the solution with 2 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.4 Barium standard stock solution (1000 µg/mL Ba) - Prepare by dissolving exactly 1.5163 g $BaCl_2$ (previously dried for two hours at 250 EC) in a mixture of 10 mL of reagent water and 1 mL 50% HCl. Add 10.0 mL 50% HCl and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.5 Beryllium standard stock solution (1000 µg/mL Be) - Prepare by dissolving exactly 19.6463 g of **undried** $BeSO_4 \cdot 4H_2O$ in reagent water. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

CAUTION: Drying of beryllium salts can lead to a toxic inhalation hazard.

7.9.6 Boron standard stock solution (1000 µg/mL) - Prepare by dissolving exactly 5.716 g of undried, anhydrous H_3BO_3 in reagent water. Dilute to volume in a 1-L volumetric flask with reagent water. The use of a non-glass volumetric flask is recommended in order to avoid boron contamination from glassware. Transfer immediately to an appropriate container for storage.

7.9.7 Cadmium standard stock solution (1000 µg/mL Cd) - Prepare by dissolving exactly 1.1423 g CdO in a minimum amount of 50% HNO_3. Apply heat to aid in dissolution. Following equilibration to ambient temperature, add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.8 Calcium standard stock solution (1000 µg/mL Ca) - Prepare by suspending exactly 2.4969 g $CaCO_3$ (previously dried for one hour at 180 EC) in reagent water. Dissolve cautiously with a minimum amount of 50% HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.9 Chromium standard stock solution (1000 µg/mL Cr) - Prepare by dissolving exactly 1.9231 g CrO_3 in reagent water. Following dissolution, acidify the

solution with 10 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.10 Cobalt standard stock solution (1000 µg/mL Co) - Prepare by dissolving exactly 1.000 g of cobalt metal in a minimum amount of 50% HNO_3. Add 10.0 mL 50% HCl and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.11 Copper standard stock solution (1000 µg/mL Cu) - Prepare by dissolving exactly 1.2564 g CuO in a minimum amount of 50% HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.12 Iron standard stock solution (1000 µg/mL Fe) - Prepare by dissolving exactly 1.4298 g Fe_2O_3 in a warm mixture of 20 mL 50% HCl and 2 mL of concentrated HNO_3. Following equilibration to ambient temperature, add an additional 5.0 mL of concentrated HNO_3. Dilute the solution to volume in a 1-L volumetric flask with reagent water.

7.9.13 Lead standard stock solution (1000 µg/mL Pb) - Prepare by dissolving exactly 1.5985 g $Pb(NO_3)_2$ in a minimum amount of 50% HNO_3. Add 10 mL 50% HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.14 Lithium standard stock solution (1000 µg/mL Li) - Prepare by dissolving exactly 5.3248 g lithium carbonate in a minimum amount of 50% HCl. Dilute to volume in a 1-L volumetric flask with reagent water.

7.9.15 Magnesium standard stock solution (1000 µg/mL Mg) - Prepare by dissolving exactly 1.6584 g MgO in a minimum amount of 50% HNO_3. Add 10.0 mL 50% HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.16 Manganese standard stock solution (1000 µg/mL Mn) - Prepare by dissolving exactly 1.000 g of manganese metal in an acid mixture of 10 mL concentrated HCl and 1 mL concentrated HNO_3. Dilute to volume in a 1-L volumetric flask with reagent water.

7.9.17 Mercury standard stock solution (1000 µg/mL Hg) - Prepare by dissolving exactly 1.354 g of **undried** $HgCl_2$ in reagent water. Add 50.0 mL concentrated HNO_3 and dilute to volume in 1-L volumetric flask with reagent water.

CAUTION: Drying of mercury salts can lead to a toxic inhalation hazard.

7.9.18 Molybdenum standard stock solution (1000 µg/mL Mo) - Prepare by dissolving exactly 1.7325 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in reagent water and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.19 Nickel standard stock solution (1000 µg/mL Ni) - Prepare by dissolving exactly 1.000 g of nickel metal in 10.0 mL of hot, concentrated HNO_3. Following equilibration to ambient temperature, dilute the solution to volume in a 1-L volumetric flask with reagent water.

7.9.20 Phosphate standard stock solution (1000 µg/mL P) - Prepare by dissolving exactly 4.3937 g of anhydrous KH_2PO_4 in reagent water. Dilute to volume in a 1-L volumetric flask with reagent water.

7.9.21 Potassium standard stock solution (1000 µg/mL K) - Prepare by dissolving exactly 1.9069 g KCl (dried to a constant weight at 110EC) in reagent water. Dilute to volume in a 1-L volumetric flask with reagent water.

7.9.22 Selenium standard stock solution (1000 µg/mL Se) - Prepare by dissolving exactly 1.6332 g of **undried** H_2SeO_3 in reagent water and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.23 Silica standard stock solution (1000 µg/mL SiO_2) - Prepare by dissolving exactly 2.964 g of **undried** $(NH4)_2SiF6$ in 200 mL of 1:20 HCl. Heat solution to 85 ^oC to aid in dissolution. After allowing the solution to equilibrate to ambient temperature, dilute to volume in a 1-L volumetric flask with reagent water. Transfer immediately to an appropriate container for storage. Protect standard from light during storage.

7.9.24 Silver standard stock solution (1000 µg/mL Ag) - Prepare by dissolving exactly 1.5748 g of $AgNO_3$ in a reagent water mixture of 10 mL concentrated HNO_3. Dilute to volume in a 1-L volumetric flask with reagent water. Protect standard from light during storage.

7.9.25 Sodium standard stock solution (1000 µg/mL Na) - Prepare by dissolving exactly 2.5419 g of NaCl in reagent water. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.26 Strontium standard stock solution (1000 µg/mL Sr) - Prepare by dissolving exactly 2.4154 g of $Sr(NO_3)_2$ in a 1-L volumetric flask containing 10 mL of concentrated HCl and 700 mL of reagent water. Dilute to volume with reagent water.

7.9.27 Thallium standard stock solution (1000 µg/mL Tl) - Prepare by dissolving exactly 1.3034 g $TlNO_3$ in reagent water. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.28 Tin standard stock solution (1000 µg/mL Sn) - Prepare by dissolving exactly 1.000 g Sn shot in 200 mL of 50% HCl. Apply heat to aid in dissolution. After allowing the solution to equilibrate to ambient temperature, dilute to volume in a 1-L volumetric flask with 50% HCl.

7.9.29 Vanadium standard stock solution (1000 µg/mL V) - Prepare by dissolving exactly 2.2957 g NH_4VO_3 in a minimum amount of concentrated HNO_3. Apply heat to aid in dissolution. After allowing the solution to equilibrate to ambient temperature, add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.30 Zinc standard stock solution (1000 µg/mL Zn) - Prepare by dissolving exactly 1.2447 g ZnO in a minimum amount of dilute HNO_3. Add 10.0 mL of concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.9.31 Yttrium internal standard stock solution (1000 µg/mL Y) - Prepare by dissolving exactly 4.3081 g $Y(NO_3)_3 \cdot 6H_2O$ in a minimum amount of concentrated HNO_3. Add 10.0 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.10 If the determination of one or more metals using a non-aqueous solvent is required, then all standards and quality control samples must be prepared on a weight/weight basis in the non-aqueous solvent since the density of non-aqueous solvents is not uniform. Standards and quality control materials containing organometallic materials that are soluble in non-aqueous solvents are available from a variety of vendors.

7.11 Working-level standard solutions and blanks

NOTE: Following the preparation of all intermediate- and working-level standard solutions, blanks, and QC standards (Sec. 9.0) immediately transfer to an appropriate container for storage. For all intermediate and working standards, especially low-level standards (i.e., < 1 µg/mL metal), the stability must be demonstrated prior to use. Working-level standards should be prepared as needed, recognizing the fact that low-level metal standards can degrade rapidly over time (Refer to Sec. 10.6 for guidance on determining the integrity of standards).

7.11.1 Mixed-calibration standard solutions — Prepare by combining proper volumes of the standard stock solutions in 100-mL volumetric flasks. Add the appropriate types and volumes of acids so that the matrices of the standards are matched, relative to those of the sample digestates. Store all mixed-calibration standards in an appropriate container and protect from light. Prior to preparing the mixed standards, each standard stock solution should be analyzed, separately, in order to determine possible spectral interferences and/or the presence of impurities. Standards which interfere with another analyte, or which are contaminated with another analyte, may not be included in the same calibration standard as that analyte. Refer to Table 2 for recommendations in selecting the most appropriate stock standards (Sec. 7.9) to combine for the preparation of working-level, mixed–calibration standards.

NOTE: Care should be taken when preparing the calibration standards to ensure that the elements are compatible and stable when mixed together.

NOTE: Depending on the acid combination of the resulting mixed-standard solution, the formation of a precipitate may occur upon addition of the silver standard stock solution. If this happens, add 15 mL of reagent water and apply heat to the volumetric flask until the solution clears. Equilibrate the flask to ambient temperature following dissolution and dilute to volume with reagent water. For such an acid combination, the silver concentration should be limited to 2 mg/L. Silver is stable under these conditions in a water matrix for 30 days if protected from the light. Higher concentrations of silver necessitate the use of additional HCl.

7.11.2 Blanks - Two types of blanks are necessary for the analysis of samples prepared by any method, other than Method 3040: (1) the calibration blank is used in establishing the analytical curve; and (2) the method blank contains all of the exact same reagents, and in the same proportions, as those used for the processing of samples, and

is thus used to identify possible contamination resulting from either the reagents (namely, acids) or equipment (including even filters) used during sample processing.

7.11.2.1 Calibration blank - Prepare by acidifying reagent water using the same combination and concentrations of acids used in the preparation of the matrix-matched calibration standards (Sec. 7.11.1). Prepare a sufficient quantity, such that it may be used to flush the system in between standards and samples. The calibration blank will also be used for all initial calibration blank (ICB) and continuing calibration blank (CCB) determinations.

7.11.2.2 Method blank - Prepare by processing either a volume of reagent water equal to that used for actual aqueous samples, or, otherwise, a clean, empty container, equivalent to that used for actual solid samples through all of the preparatory and instrument determination steps used for making ICP-OES determinations in samples. These steps may include, but are not limited to, pre-filtering, digestion, dilution, filtering, and analysis (refer to Sec. 9.7.1).

7.11.3 Initial calibration verification (ICV) standard - Prepare by combining compatible metals from standard stock solution sources that differ from those used for the preparation of the calibration standards (Sec. 7.11.1); or otherwise, purchase an already-prepared, second-source reference material from a different commercial lot or vendor. The ICV should be prepared so as to contain metal concentrations equal or nearly equivalent to the midpoint concentration level of the calibration curve (see Sec. 10.8 for use).

7.11.4 Continuing calibration verification (CCV) standard - Prepare using the same acid matrix and stock standards employed when preparing the calibration standards. The CCV should be prepared so as to contain metal concentrations equal or nearly equivalent to the midpoint concentration of the calibration curve (see Sec. 10.8 for use).

7.12 SIC solutions - The SIC solutions must be used regardless of whether or not interelement corrections are applied. They evaluate both potential spectral interferences and the accuracy of any correction equations.

7.12.1 Individual element SIC solutions - Individual element SIC solutions are used to evaluate possible spectral interferences and to set interelement corrections if necessary. A solution of each element is prepared at the highest concentration in the linear range likely to be observed in samples. The acid strength should be equivalent to that of the calibration standards. See section 9.9.1 for use of the individual element SIC solutions. SIC solutions should be tested to verify that they are not contaminated with elements of interest. The verification of purity can be done by analysis using an alternate technology, such as ICP-MS. For ICP-OES instruments with solid-state detectors, the verification might also be done by examining alternate wavelengths. If the SIC solutions are purchased ready-made, the vendor should provide details of any contaminants. In some cases, it may not be possible to obtain solutions completely free of contaminants, in which case the known, verified concentration can be subtracted from the instrument result before assessing any interferences.

7.12.2 Mixed element SIC solution - The mixed element SIC solution is used as an ongoing daily check of freedom from spectral interferences. The mixed element SIC

solution contains the following elements and is made up in an acid solution equivalent to the calibration standards. See Sec. 9.9.2 for use of the mixed element SIC solution. As for the single element solutions described in 7.12.1 known and documented contaminants are subtracted from the observed values in the mixed element SIC check.

Mixed element SIC solution: Aluminum, 500mg/L; Calcium, 500mg/L; Iron, 200mg/L; Magnesium, 500mg/L

8.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

See Chapter Three, Inorganic Analytes, for sample collection and preservation instructions.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over those criteria given in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800 for QC procedures to ensure the proper operation of the various sample preparation techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3005, 3010, 3015, 3031, 3040, 3050, 3051, 3052, 7000, and 6800.

9.3 Instrument Detection Limits

IDLs are useful means to evaluate the instrument noise level and response changes over time for each analyte from a series of reagent blank analyses to obtain a calculated concentration. They are not to be confused with the lower limit of quantitation, nor should they be used in establishing this limit. It may be helpful to compare the calculated IDLs to the established lower limit of quantitation, however, it should be understood that the lower limit of quantitation needs to be verified according to the guidance in Sec. 9.8. IDLs in µg/L can be estimated as the mean of the blank results plus three times the standard deviation of 10 replicate analyses of the reagent blank solution. (Use zero for the mean if the mean is negative). Each measurement should be performed as though it were a separate analytical

sample (i.e., each measurement must be followed by a rinse and/or any other procedure normally performed between the analysis of separate samples). IDLs should be determined at least once using new equipment, after major instrument maintenance such as changing the detector, and/or at a frequency designated by the project. An instrument log book should be kept with the dates and information pertaining to each IDL performed.

9.4 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination by generating data of acceptable precision and bias for target analytes in a clean matrix. If an autosampler is used to perform sample dilutions, before using the autosampler to dilute samples, the laboratory should satisfy itself that those dilutions are of equivalent or better accuracy than is achieved by an experienced analyst performing manual dilutions. It is recommended that the laboratory should repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made.

9.5 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment that come into direct contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are digested and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If an interference is observed that would prevent the determination of the target analyte, determine the source and eliminate it, if possible, before processing the samples. The method blank should be carried through all stages of sample preparation and instrument determination procedures. When new reagents or chemicals are received, the laboratory should monitor the preparation and/or analysis blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks need to be prepared for each set of reagents.

9.6 Linear range

The linear range establishes the highest concentration that may be reported without diluting the sample. Following calibration, the laboratory may choose to analyze a standard at a higher concentration than the high standard in the calibration. The standard must recover within 10% of the true value, and if successful, establishes the linear range. The linear range standards must be analyzed in the same instrument run as the calibration they are associated with (i.e., on a daily basis) but may be analyzed anywhere within that run. If a linear range standard is not analyzed for any specific element, the highest standard in the calibration becomes the linear range.

NOTE: Many of the alkali- and alkaline-earth metals have second-order response curves due to ionization and self-absorption effects. These effects can be minimized by using an easily ionized element in excess in the internal standard or standards themselves. Lithium or cesium are good candidates. Second-order calibration curves may be used for alkali or alkaline earth metals if the instrumentation and software can accommodate them. However, the effective range must be checked and the second-order curve fit should have a correlation coefficient of 0.995 or better. Third-order calibration fits are not acceptable. Second-order response curves should be revalidated and recalculated at least every six months. These curves are much more sensitive to changes in

operating conditions than the first-order curves and should be checked whenever there have been moderate equipment changes.

9.7 Sample QC for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, and sensitivity). At a minimum, this should include the analysis of QC samples including a method blank, a matrix spike (MS), a laboratory control sample (LCS), and a duplicate sample in each analytical batch. All method blanks, LCSs, MS samples, and duplicate samples should be subjected to the same preparatory and instrumentation procedures (Sec. 11.0) as those used on actual samples.

9.7.1 For each batch of samples analyzed, at least one method blank must be carried throughout the entire sample preparation and instrument determination process, as described in Chapter One. The importance of the method blank is to aid in identifying when and/or if sample contamination is occurring. The method blank is considered to be acceptable if it does not contain the target analytes at concentration levels that exceed the acceptance limits defined in Chapter One or in the project-specific DQOs. The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" is not reliable because it is based on a single method blank value rather than a statistically determined blank concentration.

Blanks are generally considered to be acceptable if target analyte concentrations are less than ½ the lower limit of quantitation (LLOQ) or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., targets are not present in samples or sample concentrations **are ≥10X** the blank). Other criteria may be used depending on the needs of the project.

If the method blank fails to meet the necessary acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples associated with the method blank must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples. If the method blank results do not meet the acceptance criteria and reanalysis is not practical, then the laboratory should report the sample results along with the method blank results and provide a discussion of the potential impact of the contamination on the sample results. However, if an analyte of interest is found in a sample in the batch near its concentration confirmed in the blank, the presence and/or concentration of that analyte should be considered suspect and may require qualification. Refer to Chapter One for additional guidance regarding the proper protocol when analyzing method blanks.

9.7.2 Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one matrix spike/matrix spike duplicate (MS/MSD) pair for each batch of samples processed, as described in Chapter One. An MS/MSD pair is used to document the bias and precision of a method in a given sample matrix. The decision on whether to prepare and analyze duplicate samples or an MS/MSD pair must be based on knowledge of the samples in the analysis batch. If samples are expected to contain target analytes, laboratories may choose to use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, the laboratories should use an MS/MSD pair.

MS/MSD samples should be spiked with each target element at the project-specific action levels, or, when lacking project-specific action levels, between the low- and mid-level standards, as appropriate. Acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses, for each matrix type being analyzed. However, historically derived acceptance limits must not exceed ± 25% recovery of the target element spike values for accuracy, and ≤ 20 relative percent difference (RPD) for precision. In the absence of historical data, MS/MSD acceptance limits should be set at ± 25% recovery and ≤ 20 RPD. Refer to Sec. 1.1.4 of Chapter One for further guidance. If the bias and precision indicators in an analytical batch fail to meet the acceptance criteria, then the interference test discussed in Sec. 9.11 should be performed. Refer to the definitions of bias and precision, in Chapter One, for the proper data reduction protocols.

NOTE: If the background sample concentration is very low or non-detect, a spike of greater than 5 times the background concentration is still acceptable.

$$RPD = \frac{|D_1 - D_2|}{\left(\frac{|D_1 + D_2|}{2}\right)} \times 100$$

To assess data precision with duplicate analyses, it is preferable to use a high concentration field sample to prepare unspiked laboratory duplicates for metals analyses.

Calculate the RPD between duplicate or MS determinations as follows:
where:

RPD = relative percent difference
D_1 = MS or first sample analysis value
D_2 = MSD or duplicate sample analysis value

9.7.3 At least one LCS should be prepared and analyzed with each batch of analytical samples processed, as described in Chapter One. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS should be spiked at the same levels and using the same spiking materials as the corresponding MS/MSD (see above Sec. 9.7.2). When the results of the MS analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can acceptably perform the analysis in a clean matrix.

LCS acceptance criteria should be set at laboratory-derived limits, developed through the use of historical analyses. However, historically derived acceptance limits must not exceed ± 20% of the target element spike values. In the absence of historical data, LCS acceptance limits should be set at ± 20%. If the result of an LCS does not meet the established acceptance criteria, it should be re-analyzed once. If still unacceptable, then all samples after the last acceptable method blank must be re-prepared and re-analyzed, along with all other appropriate analysis batch QC samples.

9.7.4 Reference materials containing known amounts of target elements are recommended when appropriately similar mediums of interest are available as one type of QC after appropriate sample preparation. The reference material may be used as the LCS. For soil reference materials, the manufacturers' established acceptance criterion

should be used. For solid reference materials, ± 20% recovery (see Sec. 9.7.3) of the reported manufacturers' target element values may not be achievable. Refer to Chapters One and Three for additional information.

9.8 Lower Limit of Quantitation check standard

9.8.1 The laboratory should establish the LLOQ as the lowest point of quantitation which, in most cases, is the lowest concentration in the calibration curve. The LLOQ is initially verified by the analysis of at least 7 replicate samples, spiked at the LLOQ and processed through all preparation and analysis steps of the method. The mean recovery and relative standard deviation of these samples provide an initial statement of precision and accuracy at the LLOQ. In most cases the mean recovery should be +/- 35% of the true value and RSD should be $\leq$ 20%. In-house limits may be calculated when sufficient data points exist. Monitoring recovery of LLOQ over time is useful for assessing precision and bias. Refer to a scientifically valid and published method such as Chapter 9 of Quality Assurance of Chemical Measurements (Taylor 1987) or the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs (http://water.epa.gov/scitech/methods/cwa/det/index.cfm) for calculating precision and bias for LLOQ.

9.8.2 Ongoing LLOQ verification, at a minimum, is on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with clean control material (e.g., reagent water, method blanks, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix that is free of target compounds. Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated project-specific requirements.

9.9 Spectral interference checks

Two types of SIC checks are used. Individual element SIC checks are performed when the instrument is initially setup, and periodically (at least once every 6 months) thereafter. The mixed element SIC solution is used daily to check that the instrument is free from interference from elements typically observed in high concentration and to check that and interference corrections applied are still valid.

9.9.1 Single element interference checks - At a minimum, single element SIC checks must be performed for the following elements:

Aluminum 500mg/L; Boron 50mg/L, Barium, 50mg/L, Calcium 500mg/L; Copper 50mg/L; Iron 200mg/L; Magnesium 500mg/L; Manganese 50mg/L; Molybdenum 20mg/L; Sodium 1000mg/L; Nickel 20mg/L; Selenium 20mg/L; Silicon 200mg/L; Tin 20mg/L; Vanadium 20mg/L; Zinc 20mg/L

The absolute value of the concentration observed for any unspiked analyte in the single element SIC checks must be less than two times the analytes' LLOQ. The concentration of the SIC checks are suggested, but become the highest concentration allowed in a sample analysis and cannot be higher than the highest established linear range. Samples with concentrations of elements higher than the SIC check must be diluted until the concentration is less than the SIC check solution. Note that reanalysis of a diluted sample is required even if the high concentration element is not required to be

reported for the specific sample, since the function of the SIC check is to evaluate spectral interferences on other elements.

The single element SIC checks are performed when the instrument is setup and periodically (at least once every 6 months) thereafter.

9.9.2 Mixed element interference check - The mixed element SIC solution (see section 7.12.2) is analyzed at least once per day, immediately after the initial calibration. The concentration measured for any target analytes must be less than +/- the LLOQ. If this criterion is not met then sample analysis may not proceed until the problem is corrected, or alternatively the LLOQ may be raised to twice the concentration observed in the SIC solution. The only exceptions are those elements that have been demonstrated to be contaminants in the SIC solutions (see Section 7.12.1). These may be present up to the concentration documented plus the LLOQ.

9.10 It is recommended that the laboratory adopt additional QA practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze reference materials and participate in relevant performance evaluation (PE) studies.

9.11 If less than acceptable bias and precision data are generated for the MS(s), the additional QC protocols in sections 9.11.1 and/or 9.11.2 should be performed prior to reporting concentration data for the elements in this method. At a minimum these tests should be performed with each batch of samples prepared/analyzed with corresponding unacceptable data quality results. If matrix interference effects are confirmed, then an alternative test method should be considered or the current test method modified, so that the analysis is not affected by the same interference. The use of a standard-addition analysis procedure may also be used to compensate for this effect (refer to Method 7000).

9.11.1 Dilution test

If the analyte concentration is within the linear range of the instrument and sufficiently high (minimally, a factor of 25 times greater than the LLOQ), an analysis of a 1:5 dilution should agree to within ± 20% of the original determination. If not, then a chemical or physical interference effect should be suspected. The MS is often a good choice of sample for the dilution test, since reasonable concentrations of most analytes are present. Elements that fail the dilution test are reported as estimated values.

CAUTION: If spectral overlap is suspected, then the use of computerized compensation, an alternate wavelength, or comparison with an alternate method is recommended.

9.11.2 Post-digestion MS

If a high concentration sample is not available for performing the dilution test, then a post-digestion MS should be performed. The test only needs to be performed for the specific elements that failed original MS limits, and only if the spike concentration added was greater than the concentration determined in the unspiked sample. Following preparation, which may include, but is not limited to, pre-filtration, digestion, dilution and filtration, an aliquot, or dilution thereof, should be obtained from the final aqueous, unspiked-analytical sample, and spiked with a known quantity of target elements. The

spike addition should be based on the indigenous concentration of each element of interest in the sample. The recovery of the post-digestion MS should fall within a ± 25 % acceptance range, relative to the known true value, or otherwise within the laboratory-derived acceptance limits. If the post-digestion MS recovery fails to meet the acceptance criteria, the sample results must be reported as estimated values.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Before using this procedure for quantitation, ensure that initial demonstration of performance data is available for viewing. Such data must document:

- The selection criteria for background correction points;
- analytical dynamic ranges, including the applicable equations, and upper limits of ranges;
- IDLs and method LLOQs; and
- The determination and verification of interelement correction equations, or other routines for correcting spectral interferences. These data must be generated using the same instrument, operating conditions, and calibration routine to be used for sample analysis. The data must be kept on file and available for review by the data user or auditor.

10.2 Set up the instrument using the appropriate operating conditions. Follow the instructions provided by the instrument manufacturer unless other conditions provide similar or better results. Specific wavelengths for use in quantitation should be selected from the manufacturer’s instructions. Other wavelengths may be substituted if they can provide the needed sensitivity and are corrected for spectral interferences. Because of differences among various makes and models of spectrometers, specific instrument operating conditions cannot be provided. The instrument and operating conditions utilized for determination must be capable of providing data of acceptable quality based on the specific program and end user. Operating conditions for aqueous solutions usually vary from:

- 1100-1500-watts forward power;
- 14-18-mm viewing height;
- 15-19-L/min argon-coolant flow;
- 0.6-1.5-L/min argon-nebulizer flow; and
- 1.0-1.8-mL/min sample-pumping rate; with a 1-min pre-flush time and measurement time near 1 sec/wavelength peak for sequential instruments and 10 sec/sample for simultaneous instruments.

One recommended way in which to achieve repeatable interference correction factors is to adjust the argon-aerosol flow to reproduce the Cu/Mn intensity ratio at the wavelengths 324.754 nm and 257.610 nm.

10.3 Plasma optimization

Optimize the plasma operating conditions prior to use of the instrument. The purpose of plasma optimization is to provide a maximum signal-to-background ratio for some of the least sensitive elements in the analytical array. The use of a mass-flow controller to regulate the nebulizer gas flow or source optimization software greatly facilitates the procedure. This routine

is not needed on a daily basis, but only is necessary when first setting up a new instrument or following a change in operating conditions. The following procedure is recommended; otherwise follow the manufacturer's guidelines.

10.3.1 Ignite the radial plasma and select an appropriate incident RF power. Allow the instrument to become thermally stable before beginning; approximately 30 - 60 minutes of operation. Optimize the ICP per manufacturer's instructions or alternatively by the following procedure: While aspirating a 1000-µg/L solution of yttrium, follow the instrument manufacturer's instructions and adjust the aerosol carrier gas-flow rate through the nebulizer, so that a definitive blue emission region of the plasma extends approximately 5 - 20 mm above the top of the load coil. Record the nebulizer gas-flow rate or pressure setting for future reference. The yttrium solution can also be used for coarse optical alignment of the torch, by observing the overlay of the blue light over the entrance slit to the optical system. If yttrium is an analyte of interest in samples, be aware it may take some time to rinse out 1000 µg/L yttrium solution.

10.3.2 After establishing the nebulizer gas-flow rate, determine the solution-uptake rate of the nebulizer in mL/min, by aspirating a known volume of a calibration blank for a period of at least one minute. Divide the volume (mL) aspirated by the time (min) and record the uptake rate. Set the peristaltic pump to deliver this rate.

10.3.3 Profile the instrument per manufacturer's directions to align it optically, as it will be used during analysis.

10.3.4 Complete the following procedure for vertical optimization or follow manufacturer's directions:

NOTE: This procedure can be used for both vertical and horizontal optimization.

Aspirate a solution containing 10 µg/L of several selected elements. As, Se, Tl, and Pb are the least sensitive of the elements and most in need of optimization. However, other elements may be used, based on the professional judgment of the analyst (V, Cr, Cu, Li and Mn have also been used with success). Collect intensity data at the wavelength peak for each analyte at 1mm intervals from 14 – 18 mm above the load coil. (This region of the plasma is referred to as the "analytical zone".) Repeat this process using the calibration blank. Determine the net signal-to-blank-intensity ratio for each analyte for each viewing height setting. Choose the height for viewing the plasma that provides the best net intensity ratios for the elements analyzed or the highest intensity ratio for the least sensitive element.

For optimization in the axial mode, follow the instrument manufacturer's instructions.

10.3.5 The instrument operating conditions finally selected as being optimum should provide the lowest reliable IDLs.

10.3.6 If the instrument operating conditions, such as incident power or nebulizer gas-flow rate, are changed, or if a new torch injector with a different orifice internal diameter is installed, then the plasma and viewing height should be re-optimized.

10.3.7 After completing the initial optimization of operating conditions, and before analyzing samples, establish and initially verify an interelement spectral interference correction routine to be used during sample analysis. A general description of spectral interferences and the analytical specifications for background correction, in particular, are discussed in Sec. 4.0. Directions for verification of freedom from interference are given in Sections 7.12 and 9.9. The criterion for determining that an interelement spectral interference is present is an apparent positive or negative concentration for the analyte that falls beyond ± the LLOQ from zero. The upper control limit is the analyte LLOQ. Once established, verify the entire routine at least once every six months. Only a portion of the correction routine must be verified more frequently or on a daily basis. Initial and periodic verifications of the routine should be kept on file.

10.3.8 Before daily calibration, and after the instrument warm-up period, the nebulizer gas-flow rate must be reset to the determined optimized flow. If a mass-flow controller is being used, it should be set to the recorded optimized flow rate. In order to maintain valid spectral interelement correction routines, the nebulizer gas-flow rate should be the same (< 2% change) from day to day.

10.4 For operation with organic solvents, the use of the auxiliary argon inlet is recommended, as is the use of solvent-resistant tubing, increased plasma (coolant) argon flow, decreased nebulizer flow, and increased radio frequency (RF) power, in order to obtain a robust plasma, stable operating conditions, and precise measurements.

10.5 At a minimum, the elements required for the project plus any required for interference correction must be calibrated. Recommended wavelengths for the analytes in Sec. 1.1 should be obtained from the instrument manufacturer. Flush the system in between each standard and sample using the rinse blank. The rinse time needs to be sufficient to ensure that analytes present at the linear range are effectively cleaned out prior to analysis of the subsequent sample. Use the average of at least three integrations for both calibration standard and sample analyses.

10.6 Calibration standards should be prepared on an as-needed basis unless stability warrants preparing fresh daily, (or each time a batch of samples is analyzed). If the ICV standard is prepared daily and the results of the ICV analyses meet the acceptance criteria, then the calibration standards do not need to be prepared daily and may be prepared and stored for as long as the calibration standard viability can be verified through the use of the ICV. If the ICV fails to meet the acceptance criteria, trouble shoot the situation, and then prepare a new set of calibration standards if needed and recalibrate the instrument.

10.7 A calibration curve must be analyzed daily. The instrument may be calibrated using a single point standard and a calibration blank (ICB) or a multipoint calibration curve. If a multipoint curve is used a minimum of three standards are required and the correlation coefficient (r) should be $\geq$ 0.995 or the coefficient of determination (r^2) should be $\geq$ 0.990. Relative Standard Error may be used as an alternative to r or r^2 and should be $\leq$ 20%. If a multipoint calibration is used the low standard must be at or below the LLOQ.

NOTE: Inversely weighted linear regressions are recommended in order to minimize curve fitting errors at the low end of the calibration curve.

10.8 After the calibration is completed it is verified using several checks.

10.8.1 Initial Calibration Verification - The ICV is a standard prepared from a separate source than the initial calibration standards. It is analyzed at approximately the mid-level of the calibration and serves as a check that the initial calibration standards are at the correct concentrations. The acceptance range is 90-110% of the true value.

10.8.2 Low-level readback or verification - For a multi-point calibration, the low-level standard should quantitate to within 80-120% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at the LLOQ is analyzed and should recover within 80-120% of the true value.

10.8.3 Mid-level readback or verification - For a multi-point calibration, the mid-level standard should quantitate to within 90-110% of the true value. For a single point calibration, a standard from the same source as the calibration standard and at the mid-point of the linear range is analyzed and should recover within 90-110% of the true value.

10.8.4 Initial Calibration blank - If a multi-level calibration is used, an ICB is analyzed immediately after the calibration (or after the ICV) and must not contain target analytes above half the LLOQ. If a single point calibration is used, the calibration is forced through the ICB, but a second ICB is analyzed as a check and must not contain target analytes above half the LLOQ. If the ICB consistently has target analyte concentrations greater than half the LLOQ, the LLOQ should be re-evaluated.

10.8 5 Verify the ongoing validity of the calibration curve after every 10 samples, and at the end of each analysis batch run, through the analysis of a CCV standard (Sec. 7.11.4 and a CCB (Sec. 7.11.2.1). For the curve to be considered valid the analysis result of the CCV standard must be within ± 10% of its true value and the CCB must not contain target analytes above the LLOQ. If the calibration cannot be verified, sample analysis must be discontinued, the cause of the problem determined and the instrument recalibrated. All samples following the last acceptable CCV standard must be reanalyzed Flow-injection systems may be used as long as they can meet the performance criteria of the method.

NOTE: During the course of an analytical run, the instrument may be recalibrated to correct for instrument drift. A recalibration must then be followed immediately by a new analysis of a CCV and CCB before any further samples may be analyzed.

11.0 PROCEDURE

11.1 Preliminary treatment of most samples is necessary because of the complexity and variability of sample matrices. Groundwater samples which have been pre-filtered and acidified will not need acid digestion. Samples which are not digested must either use an internal standard or be matrix-matched with the standards (i.e., acid concentrations should match). Solubilization and digestion procedures are presented in Chapter Three, Inorganic Analytes.

11.2 Profile and calibrate the instrument according to the instrument manufacturer's recommended procedures, using the typical mixed-calibration standard solutions described in Sec. 7.11.1. Prepare the calibration curve as detailed in Sec. 10.7. Flush the system between each standard using the calibration blank (Sec. 7.11.2.1), or as the manufacturer recommends. In order to reduce random error, use the average intensity of multiple exposures for both standardization and sample analysis.

11.3 For all analytes and determinations, the laboratory must analyze an ICV (Secs 7.11.3 and 10.8.1) and a CCV (Secs. 7.11.4 and 10.8.5) and CCB (Secs. 7.11.2.1 and 10.8.5) after every ten samples and at the end of the analysis batch run.

11.4 Analyze the samples and record the results. In between each sample or standard, rinse the system using the calibration blank solution (Sec. 7.11.2.1). Use a minimum rinse time of one minute. Each laboratory may establish a reduction in the rinse time following a suitable demonstration.

11.5 Determination of percent dry weight

When sample results are to be calculated on a dry-weight basis, a separate portion of sample for this determination should be weighed out at the same time as the portion used for analytical determination.

CAUTION: The drying oven should be contained in a hood or vented. Significant laboratory contamination may result from a heavily contaminated hazardous waste sample.

11.5.1 Immediately after weighing the sample aliquot to be digested, weigh an additional 5- to 10-g aliquot of the sample into a tared crucible. Dry this aliquot overnight at 105 °C. Allow the sample to cool in a desiccator before weighing.

11.5.2 Calculate the % dry weight as follows:

$$\%\text{ dry weight} = \frac{\text{g of dry sample}}{\text{g of sample}} \times 100$$

This oven-dried aliquot is not used for the extraction and should be appropriately disposed of once the dry weight is determined.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 If dilutions were performed, apply the appropriate dilution factors to the respective sample values. Report results up to three significant figures.

12.2 If appropriate, or required by the project or regulation for data reporting, calculate results for solids on a dry-weight basis as follows:

$$\text{Concentration}_{DW} = \frac{C \times V}{W \times S}$$

where:

$\text{Concentration}_{DW}$ = Concentration on a dry weight basis (mg/kg)
C = Digest concentration (mg/L)
V = Final volume after sample preparation (L)
W =Wet sample mass (kg)
S = % Solids/100 = % dry weight/100

13.0 METHOD PERFORMANCE

Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. <u>These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation</u>.

13.1 In an EPA round-robin study, seven laboratories applied the ICP-OES technique to water matrices spiked with various metal concentrates and acid-digested. Table 3 lists the true values, the mean reported results, and the mean percent relative standard deviations. These data are provided for guidance purposes only.

13.2 Performance data for aqueous solutions and solid samples from a multi-laboratory study are provided in Tables 5 and 6. These data are provided for guidance purposes only.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult: http://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available at: http://www.labsafety.org/FreeDocs/WasteMgmt.pdf.

16.0 REFERENCES

1. C.L. Jones, *et al*., "An Interlaboratory Study of Inductively Coupled Plasma Atomic Emission Spectroscopy Method 6010 and Digestion Method 3050," EPA-600/4-87-032, U.S. Environmental Protection Agency, Las Vegas, NV, 1987.

2. Taylor, J. K., “Quality Assurance of Chemical Measurements”, Lewis Publishers (1987).

3. Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs, Final Report, http://water.epa.gov/scitech/methods/cwa/det/index.cfm (December 28, 2007).

17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The pages to follow contain the tables, and figures referenced by this method.

TABLE 1

POTENTIAL INTERFERENCES AND ANALYTE CONCENTRATION EQUIVALENTS (mg/L) ARISING FROM INTERFERENCE AT THE 100-mg/L LEVEL

Analyte	Wavelength[c] (nm)	Interferent[a,b]									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215	--	--	--	--	--	--	0.21	--	--	1.4
Antimony	206.833	0.47	--	2.9	--	0.08	--	--	--	0.25	0.45
Arsenic	193.696	1.3	--	0.44	--	--	--	--	--	--	1.1
Barium	455.403	--	--	--	--	--	--	--	--	--	--
Beryllium	313.042	--	--	--	--	--	--	--	--	0.04	0.05
Cadmium	226.502	--	--	--	--	0.03	--	--	0.02	--	--
Calcium	317.933	--	--	0.08	--	0.01	0.01	0.04	--	0.03	0.03
Chromium	267.716	--	--	--	--	0.003	--	0.04	--	--	0.04
Cobalt	228.616	--	--	0.03	--	0.005	--	--	0.03	0.15	--
Copper	324.754	--	--	--	--	0.003	--	--	--	0.05	0.02
Iron	259.940	--	--	--	--	--	--	0.12	--	--	--
Lead	220.353	0.17	--	--	--	--	--	--	--	--	--
Magnesium	279.079	--	0.02	0.11	--	0.13	--	0.25	--	0.07	0.12
Manganese	257.610	0.005	--	0.01	--	0.002	0.002	--	--	--	--
Molybdenum	202.030	0.05	--	--	--	0.03	--	--	--	--	--
Nickel	231.604	--	--	--	--	--	--	--	--	--	--
Selenium	196.026	0.23	--	--	--	0.09	--	--	--	--	--
Sodium	588.995	--	--	--	--	--	--	--	--	0.08	--
Thallium	190.864	0.30	--	--	--	--	--	--	--	--	--
Vanadium	292.402	--	--	0.05	--	0.005	--	--	--	0.02	--
Zinc	213.856	--	--	--	0.14	--	--	--	0.29	--	--

NOTE: [a] Dashes indicate that no interference was observed even when interferents were introduced at the following levels:

Al at 1000 mg/L	Cu at 200 mg/L	Mn at 200 mg/L
Ca at 1000 mg/L	Fe at 1000 mg/L	Ti at 200 mg/L
Cr at 200 mg/L	Mg at 1000 mg/L	V at 200 mg/L

[b] The figures shown above as analyte concentration equivalents are not the actual observed concentrations. To obtain those figures, add the listed concentration to the interferent figure.

[c] Interferences will be affected by background and wavelength choice and other interferences may be present.

TABLE 2

MIXED STANDARD SOLUTIONS

Solution	Elements
I	Be, Cd, Mn, Pb, Se and Zn
II	Ba, Co, Cu, Fe, and V
III	As and Mo
IV	Al, Ca, Cr, K, Na, Ni, Li, and Sr
V	Ag[a], Mg, Sb, and Tl
VI	P

[a] See second note in Sec. 7.10.1.

TABLE 3

ICP PRECISION AND BIAS DATA[a]

Element	Sample No. 1 True Conc. (µg/L)	Sample No. 1 Mean Conc. (µg/L)	Sample No. 1 RSD[b] (%)	Sample No. 1 Accuracy[d] (%)	Sample No. 2 True Conc. (µg/L)	Sample No. 2 Mean Conc. (µg/L)	Sample No. 2 RSD[b] (%)	Sample No. 2 Accuracy[d] (%)	Sample No. 3 True Conc. (µg/L)	Sample No. 3 Mean Conc. (µg/L)	Sample No. 3 RSD[b] (%)	Sample No. 3 Accuracy[d] (%)
Be	750	733	6.2	98	20	20	9.8	100	180	176	5.2	98
Mn	350	345	2.7	99	15	15	6.7	100	100	99	3.3	99
V	750	749	1.8	100	70	69	2.9	99	170	169	1.1	99
As	200	208	7.5	104	22	19	23	86	60	63	17	105
Cr	150	149	3.8	99	10	10	18	100	50	50	3.3	100
Cu	250	235	5.1	94	11	11	40	100	70	67	7.9	96
Fe	600	594	3.0	99	20	19	15	95	180	178	6.0	99
Al	700	696	5.6	99	60	62	33	103	160	161	13	101
Cd	50	48	12	96	2.5	2.9	16	116	14	13	16	93
Co	700	512	10	73	20	20	4.1	100	120	108	21	90
Ni	250	245	5.8	98	30	28	11	93	60	55	14	92
Pb	250	236	16	94	24	30	32	125	80	80	14	100
Zn	200	201	5.6	100	16	19	45	119	80	82	9.4	102
Se[c]	40	32	21.9	80	6	8.5	42	142	10	8.5	8.3	85

NOTE: [a] Not all elements were analyzed by all laboratories.

[b] RSD = relative standard deviation.

[c] Results for Se are from two laboratories.

[d] Accuracy is expressed as the mean concentration divided by the true concentration times 100.

TABLE 4

EXAMPLE ICP-OES PRECISION AND BIAS FOR AQUEOUS SOLUTIONS

Element	Mean Concentration (mg/L)	n	RSD (%)	Accuracy (%)
Al	14.8	8	6.3	100
Sb	15.1	8	7.7	102
As	14.7	7	6.4	99
Ba	3.66	7	3.1	99
Be	3.78	8	5.8	102
Cd	3.61	8	7.0	97
Ca	15.0	8	7.4	101
Cr	3.75	8	8.2	101
Co	3.52	8	5.9	95
Cu	3.58	8	5.6	97
Fe	14.8	8	5.9	100
Pb	14.4	7	5.9	97
Mg	14.1	8	6.5	96
Mn	3.70	8	4.3	100
Mo	3.70	8	6.9	100
Ni	3.70	7	5.7	100
K	14.1	8	6.6	95
Se	15.3	8	7.5	104
Ag	3.69	6	9.1	100
Na	14.0	8	4.2	95
Tl	15.1	7	8.5	102
V	3.51	8	6.6	95
Zn	3.57	8	8.3	96

NOTE: 1. These performance values are independent of sample preparation because the labs analyzed portions of the same solutions and are provided for illustrative purposes only.
2. n = Number of measurements.3. Accuracy is expressed as a percentage of the nominal value for each analyte in acidified, multi-element solutions.

TABLE 5

EXAMPLE ICP-OES PRECISION AND BIAS FOR SOLID WASTE DIGESTS

	Spiked Coal Fly Ash (NIST-SRM 1633a)				Spiked Electroplating Sludge			
Element	Mean Conc. (mg/L)	n	RSD (%)	Bias (% AA)	Mean Conc. (mg/L)	n	RSD (%)	Bias (% AA)
Al	330	8	16	104	127	8	13	110
Sb	3.4	6	73	96	5.3	7	24	120
As	21	8	83	270	5.2	7	8.6	87
Ba	133	8	8.7	101	1.6	8	20	58
Be	4.0	8	57	460	0.9	7	9.9	110
Cd	0.97	6	5.7	101	2.9	7	9.9	90
Ca	87	6	5.6	208	954	7	7.0	97
Cr	2.1	7	36	106	154	7	7.8	93
Co	1.2	6	21	94	1.0	7	11	85
Cu	1.9	6	9.7	118	156	8	7.8	97
Fe	602	8	8.8	102	603	7	5.6	98
Pb	4.6	7	22	94	25	7	5.6	98
Mg	15	8	15	110	35	8	20	84
Mn	1.8	7	14	104	5.9	7	9.6	95
Mo	891	8	19	105	1.4	7	36	110
Ni	1.6	6	8.1	91	9.5	7	9.6	90
K	46	8	4.2	98	51	8	5.8	82
Se	6.4	5	16	73	8.7	7	13	101
Ag	1.4	3	17	140	0.75	7	19	270
Na	20	8	49	130	1380	8	9.8	95
Tl	6.7	4	22	260	5.0	7	20	180
V	1010	5	7.5	100	1.2	6	11	80
Zn	2.2	6	7.6	93	266	7	2.5	101

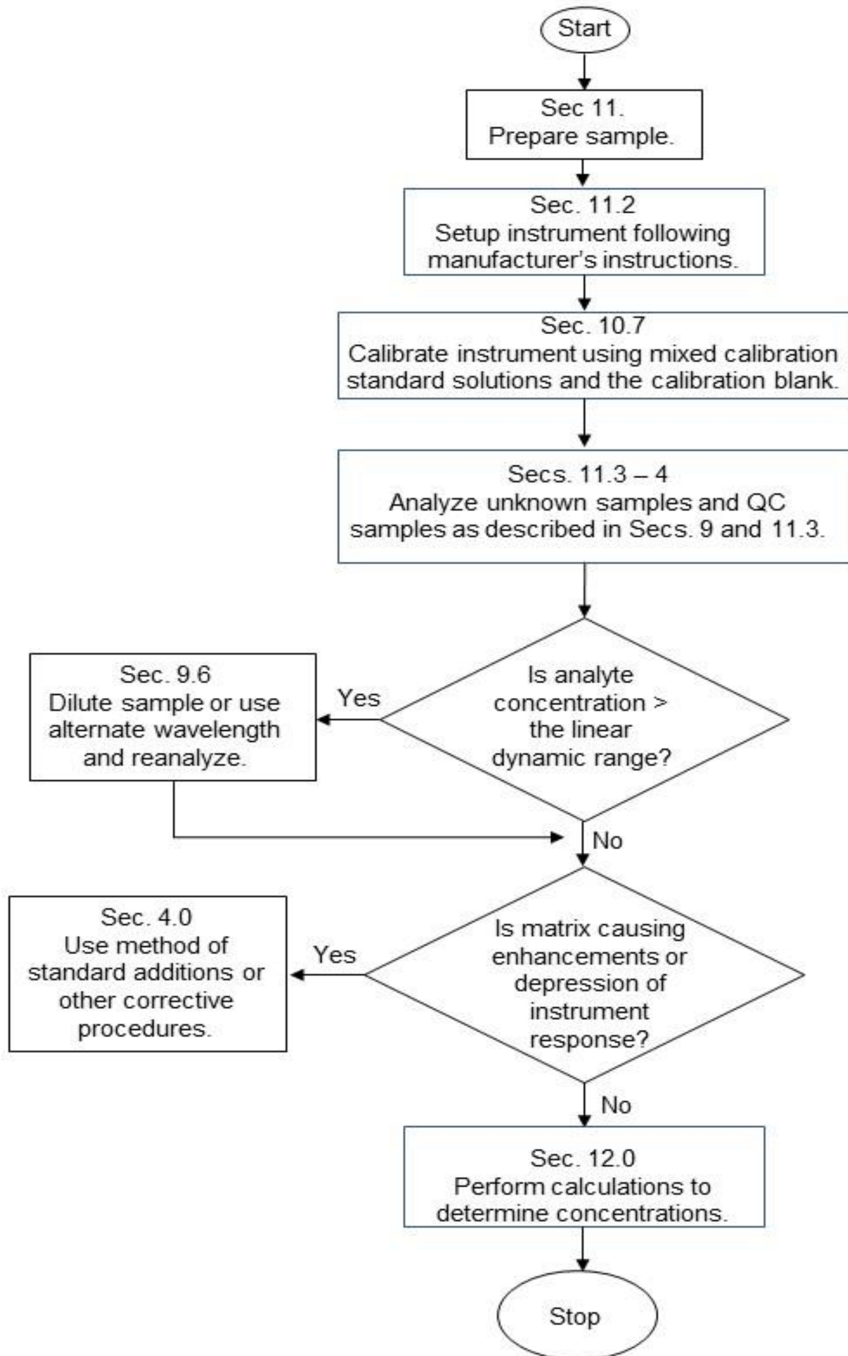
NOTE: 1. These performance values are independent of sample preparation because the labs analyzed portions of the same digests and are provided for illustrative purposes only.

2. n = Number of measurements.

3. Bias for the ICP-OES data is expressed as a percentage of atomic absorption spectroscopy (AA) data for the same digests.

FIGURE 1

INDUCTIVELY COUPLED PLASMA-OPTICAL EMISSION SPECTROMETRY



APPENDIX A

Summary of Revisions to Method 6010D (From Revision 4, July 2013):

1. The revision number was changed to 5 and the footer date updated to July 2018. A table of contents was added.

2. Sec. 9.7.2 was updated to show a reference to Chapter One, Sec 1.1.4.

3. Tables and graphics in this method were updated to be 508 compliant.

5. The ACS document in Sec. 14 was updated.

6. The reference in Sec. 15 was updated.

METHOD 3050B

ACID DIGESTION OF SEDIMENTS, SLUDGES, AND SOILS

1.0 SCOPE AND APPLICATION

1.1 This method has been written to provide two separate digestion procedures, one for the preparation of sediments, sludges, and soil samples for analysis by flame atomic absorption spectrometry (FLAA) or inductively coupled plasma atomic emission spectrometry (ICP-AES) and one for the preparation of sediments, sludges, and soil samples for analysis of samples by Graphite Furnace AA (GFAA) or inductively coupled plasma mass spectrometry (ICP-MS). The extracts from these two procedures are not interchangeable and should only be used with the analytical determinations outlined in this section. Samples prepared by this method may be analyzed by ICP-AES or GFAA for all the listed metals as long as the detecion limits are adequate for the required end-use of the data. Alternative determinative techniques may be used if they are scientifically valid and the QC criteria of the method, including those dealing with interferences, can be achieved. Other elements and matrices may be analyzed by this method if performance is demonstrated for the analytes of interest, in the matrices of interest, at the concentration levels of interest (See Section 8.0). The recommended determinative techniques for each element are listed below:

FLAA/ICP-AES		GFAA/ICP-MS
Aluminum	Magnesium	Arsenic
Antimony	Manganese	Beryllium
Barium	Molybdenum	Cadmium
Beryllium	Nickel	Chromium
Cadmium	Potassium	Cobalt
Calcium	Silver	Iron
Chromium	Sodium	Lead
Cobalt	Thallium	Molybdenum
Copper	Vanadium	Selenium
Iron	Zinc	Thallium
Lead		
Vanadium		

1.2 This method is not a total digestion technique for most samples. It is a very strong acid digestion that will dissolve almost all elements that could become “environmentally available.” By design, elements bound in silicate structures are not normally dissolved by this procedure as they are not usually mobile in the environment. If absolute total digestion is required use Method 3052.

2.0 SUMMARY OF METHOD

2.1 For the digestion of samples, a representative 1-2 gram (wet weight) or 1 gram (dry weight) sample is digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2).

2.2 For GFAA or ICP-MS analysis, the resultant digestate is reduced in volume while heating and then diluted to a final volume of 100 mL.

2.3 For ICP-AES or FLAA analyses, hydrochloric acid (HCl) is added to the initial digestate and the sample is refluxed. In an optional step to increase the solubility of some metals (see Section 7.3.1: NOTE), this digestate is filtered and the filter paper and residues are rinsed, first

with hot HCl and then hot reagent water. Filter paper and residue are returned to the digestion flask, refluxed with additional HCl and then filtered again. The digestate is then diluted to a final volume of 100 mL.

2.4 If required, a separate sample aliquot shall be dried for a total percent solids determination.

3.0 INTERFERENCES

3.1 Sludge samples can contain diverse matrix types, each of which may present its own analytical challenge. Spiked samples and any relevant standard reference material should be processed in accordance with the quality control requirements given in Sec. 8.0 to aid in determining whether Method 3050B is applicable to a given waste.

4.0 APPARATUS AND MATERIALS

4.1 Digestion Vessels - 250-mL.

4.2 Vapor recovery device (e.g., ribbed watch glasses, appropriate refluxing device, appropriate solvent handling system).

4.3 Drying ovens - able to maintain 30°C ± 4°C.

4.4 Temperature measurement device capable of measuring to at least 125°C with suitable precision and accuracy (e.g., thermometer, IR sensor, thermocouple, thermister, etc.)

4.5 Filter paper - Whatman No. 41 or equivalent.

4.6 Centrifuge and centrifuge tubes.

4.7 Analytical balance - capable of accurate weighings to 0.01 g.

4.8 Heating source - Adjustable and able to maintain a temperature of 90-95°C. (e.g., hot plate, block digestor, microwave, etc.)

4.9 Funnel or equivalent.

4.10 Graduated cylinder or equivalent volume measuring device.

4.11 Volumetric Flasks - 100-mL.

5.0 REAGENTS

5.1 Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is questionable, analyze the reagent to determine the level of impurities. The reagent blank must be less than the MDL in order to be used.

5.2 Reagent Water. Reagent water will be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One for a definition of reagent water.

5.3 Nitric acid (concentrated), HNO_3. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.4 Hydrochloric acid (concentrated), HCl. Acid should be analyzed to determine level of impurities. If method blank is < MDL, the acid can be used.

5.5 Hydrogen peroxide (30%), H_2O_2. Oxidant should be analyzed to determine level of impurities. If method blank is < MDL, the peroxide can be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be demonstrated to be free of contamination at or below the reporting limit. Plastic and glass containers are both suitable. See Chapter Three, Section 3.1.3, for further information.

6.3 Nonaqueous samples should be refrigerated upon receipt and analyzed as soon as possible.

6.4 It can be difficult to obtain a representative sample with wet or damp materials. Wet samples may be dried, crushed, and ground to reduce subsample variability as long as drying does not affect the extraction of the analytes of interest in the sample.

7.0 PROCEDURE

7.1 Mix the sample thoroughly to achieve homogeneity and sieve, if appropriate and necessary, using a USS #10 sieve. All equipment used for homogenization should be cleaned according to the guidance in Sec. 6.0 to minimize the potential of cross-contamination. For each digestion procedure, weigh to the nearest 0.01 g and transfer a 1-2 g sample (wet weight) or 1 g sample (dry weight) to a digestion vessel. For samples with high liquid content, a larger sample size may be used as long as digestion is completed.

> NOTE: All steps requiring the use of acids should be conducted under a fume hood by properly trained personnel using appropriate laboratory safety equipment. The use of an acid vapor scrubber system for waste minimization is encouraged.

7.2 For the digestion of samples for analysis by GFAA or ICP-MS, add 10 mL of 1:1 HNO_3, mix the slurry, and cover with a watch glass or vapor recovery device. Heat the sample to 95°C ± 5°C and reflux for 10 to 15 minutes without boiling. Allow the sample to cool, add 5 mL of concentrated HNO_3, replace the cover, and reflux for 30 minutes. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL of conc. HNO_3) over and over until no brown fumes are given off by the sample indicating the complete reaction with HNO_3. Using a ribbed watch glass or vapor recovery system, either allow the solution to evaporate to approximately 5 mL without boiling or heat at 95°C ± 5°C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

NOTE: Alternatively, for direct energy coupling devices, such as a microwave, digest samples for analysis by GFAA or ICP-MS by adding 10 mL of 1:1 HNO_3, mixing the slurry and then covering with a vapor recovery device. Heat the sample to 95°C ± 5°C and reflux for 5 minutes at 95°C ± 5°C without boiling. Allow the sample to cool for 5 minutes, add 5 mL of concentrated HNO_3, heat the sample to 95°C ± 5°C and reflux for 5 minutes at 95°C ± 5°C. If brown fumes are generated, indicating oxidation of the sample by HNO_3, repeat this step (addition of 5 mL concentrated HNO_3) until no brown fumes are given off by the sample indicating the complete reaction with HNO_3. Using a vapor recovery system, heat the sample to 95°C ± 5°C and reflux for 10 minutes at 95°C ± 5°C without boiling.

7.2.1 After the step in Section 7.2 has been completed and the sample has cooled, add 2 mL of water and 3 mL of 30% H_2O_2. Cover the vessel with a watch glass or vapor recovery device and return the covered vessel to the heat source for warming and to start the peroxide reaction. Care must be taken to ensure that losses do not occur due to excessively vigorous effervescence. Heat until effervescence subsides and cool the vessel.

NOTE: Alternatively, for direct energy coupled devices: After the Sec. 7.2 "NOTE" step has been completed and the sample has cooled for 5 minutes, add slowly 10 mL of 30% H_2O_2. Care must be taken to ensure that losses do not occur due to excessive vigorous effervesence. Go to Section 7.2.3.

7.2.2 Continue to add 30% H_2O_2 in 1-mL aliquots with warming until the effervescence is minimal or until the general sample appearance is unchanged.

NOTE: Do not add more than a total of 10 mL 30% H_2O_2.

7.2.3 Cover the sample with a ribbed watch glass or vapor recovery device and continue heating the acid-peroxide digestate until the volume has been reduced to approximately 5 mL or heat at 95°C ± 5°C without boiling for two hours. Maintain a covering of solution over the bottom of the vessel at all times.

NOTE: Alternatively, for direct energy coupled devices: Heat the acid-peroxide digestate to 95°C ± 5°C in 6 minutes and remain at 95°C ± 5°C without boiling for 10 minutes.

7.2.4 After cooling, dilute to 100 mL with water. Particulates in the digestate should then be removed by filtration, by centrifugation, or by allowing the sample to settle. The sample is now ready for analysis by GFAA or ICP-MS.

7.2.4.1 Filtration - Filter through Whatman No. 41 filter paper (or equivalent).

7.2.4.2 Centrifugation - Centrifugation at 2,000-3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

7.2.4.3 The diluted digestate solution contains approximately 5% (v/v) HNO_3. For analysis, withdraw aliquots of appropriate volume and add any required reagent or matrix modifier.

7.3 For the analysis of samples for FLAA or ICP-AES, add 10 mL conc. HCl to the sample digest from 7.2.3 and cover with a watch glass or vapor recovery device. Place the sample on/in the heating source and reflux at 95°C ± 5°C for 15 minutes.

NOTE: Alternatively, for direct energy coupling devices, such as a microwave, digest samples for analysis by FLAA and ICP-AES by adding 5 mL HCl and 10 mL H_2O to the sample digest from 7.2.3 and heat the sample to 95°C ± 5°C, Reflux at 95°C ± 5°C without boiling for 5 minutes.

7.4 Filter the digestate through Whatman No. 41 filter paper (or equivalent) and collect filtrate in a 100-mL volumetric flask. Make to volume and analyze by FLAA or ICP-AES.

NOTE: Section 7.5 may be used to improve the solubilities and recoveries of antimony, barium, lead, and silver when necessary. These steps are optional and are not required on a routine basis.

7.5 Add 2.5 mL conc. HNO_3 and 10 mL conc. HCl to a 1-2 g sample (wet weight) or 1 g sample (dry weight) and cover with a watchglass or vapor recovery device. Place the sample on/in the heating source and reflux for 15 minutes.

7.5.1 Filter the digestate through Whatman No. 41 filter paper (or equivalent) and collect filtrate in a 100-mL volumetric flask. Wash the filter paper, while still in the funnel, with no more than 5 mL of hot (~95°C) HCl, then with 20 mL of hot (~95°C) reagent water. Collect washings in the same 100-mL volumetric flask.

7.5.2 Remove the filter and residue from the funnel, and place them back in the vessel. Add 5 mL of conc. HCl, place the vessel back on the heating source, and heat at 95°C ± 5°C until the filter paper dissolves. Remove the vessel from the heating source and wash the cover and sides with reagent water. Filter the residue and collect the filtrate in the same 100-mL volumetric flask. Allow filtrate to cool, then dilute to volume.

NOTE: High concentrations of metal salts with temperature-sensitive solubilities can result in the formation of precipitates upon cooling of primary and/or secondary filtrates. If precipitation occurs in the flask upon cooling, do not dilute to volume.

7.5.3 If a precipitate forms on the bottom of a flask, add up to 10 mL of concentrated HCl to dissolve the precipitate. After precipitate is dissolved, dilute to volume with reagent water. Analyze by FLAA or ICP-AES.

7.6 Calculations

7.6.1 The concentrations determined are to be reported on the basis of the actual weight of the sample. If a dry weight analysis is desired, then the percent solids of the sample must also be provided.

7.6.2 If percent solids is desired, a separate determination of percent solids must be performed on a homogeneous aliquot of the sample.

8.0 QUALITY CONTROL

8.1 All quality control measures described in Chapter One should be followed.

8.2 For each batch of samples processed, a method blank should be carried throughout the entire sample preparation and analytical process according to the frequency described in Chapter One. These blanks will be useful in determining if samples are being contaminated. Refer to Chapter One for the proper protocol when analyzing method blanks.

8.3 Spiked duplicate samples should be processed on a routine basis and whenever a new sample matrix is being analyzed. Spiked duplicate samples will be used to determine precision and bias. The criteria of the determinative method will dictate frequency, but 5% (one per batch) is recommended or whenever a new sample matrix is being analyzed. Refer to Chapter One for the proper protocol when analyzing spiked replicates.

8.4 Limitations for the FLAA and ICP-AES optional digestion procedure. Analysts should be aware that the upper linear range for silver, barium, lead, and antimony may be exceeded with some samples. If there is a reasonable possibility that this range may be exceeded, or if a sample's analytical result exceeds this upper limit, a smaller sample size should be taken through the entire procedure and re-analyzed to determine if the linear range has been exceeded. The approximate linear upper ranges for a 2 gram sample size:

Ag	2,000 mg/kg
As	1,000,000 mg/kg
Ba	2,500 mg/kg
Be	1,000,000 mg/kg
Cd	1,000,000 mg/kg
Co	1,000,000 mg/kg
Cr	1,000,000 mg/kg
Cu	1,000,000 mg/kg
Mo	1,000,000 mg/kg
Ni	1,000,000 mg/kg
Pb	200,000 mg/kg
Sb	200,000 mg/kg
Se	1,000,000 mg/kg
Tl	1,000,000 mg/kg
V	1,000,000 mg/kg
Zn	1,000,000 mg/kg

NOTE: These ranges will vary with sample matrix, molecular form, and size.

9.0 METHOD PERFORMANCE

9.1 In a single laboratory, the recoveries of the three matrices presented in Table 2 were obtained using the digestion procedure outlined for samples prior to analysis by FLAA and ICP-AES. The spiked samples were analyzed in duplicate. Tables 3-5 represents results of analysis of NIST Standard Reference Materials that were obtained using both atmospheric pressure microwave digestion techniques and hot-plate digestion procedures.

10.0 REFERENCES

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TABLE 1

STANDARD RECOVERY (%) COMPARISON FOR
METHODS 3050A AND 3050B[a]

Analyte	METHOD 3050A[a]	METHOD 3050B w/option[a]
Ag	9.5	98
As	86	102
Ba	97	103
Be	96	102
Cd	101	99
Co	99	105
Cr	98	94
Cu	87	94
Mo	97	96
Ni	98	92
Pb	97	95
Sb	87	88
Se	94	91
Tl	96	96
V	93	103
Zn	99	95

[a] All values are percent recovery. Samples: 4 mL of 100 mg/mL multistandard; n = 3.

TABLE 2

PERCENT RECOVERY COMPARISON FOR METHODS 3050A AND 3050B

Analyte	Sample 4435		Sample 4766		Sample HJ		Average	
	3050A	3050B	3050A	3050B	3050A	3050B	3050A	3050B
Ag	9.8	103	15	89	56	93	27	95
As	70	102	80	95	83	102	77	100
Ba	85	94	78	95	b	b	81	94
Be	94	102	108	98	99	94	99	97
Cd	92	88	91	95	95	97	93	94
Co	90	94	87	95	89	93	89	94
Cr	90	95	89	94	72	101	83	97
Cu	81	88	85	87	70	106	77	94
Mo	79	92	83	98	87	103	83	98
Ni	88	93	93	100	87	101	92	98
Pb	82	92	80	91	77	91	81	91
Sb	28	84	23	77	46	76	32	79
Se	84	89	81	96	99	96	85	94
Tl	88	87	69	95	66	67	74	83
V	84	97	86	96	90	88	87	93
Zn	96	106	78	75	b	b	87	99

Percent Recovery[a,c]

a - Samples: 4 mL of 100 mg/mL multi-standard in 2 g of sample. Each value is percent recovery and is the average of duplicate spikes.

b - Unable to accurately quantitate due to high background values.

c - Method 3050B using optional section.

Table 3
Results of Analysis of Nist Standard Reference Material 2704
"River Sediment" Using Method 3050B (µg/g ± SD)

Element	Atm. Pressure Microwave Assisted Method with Power Control	Atm. Pressure Microwave Assisted Method with Temperature Control (gas-bulb)	Atm. Pressure Microwave Assisted Method with Temperature Control (IR-sensor)	Hot-Plate	NIST Certified Values for Total Digestion (µg/g ±95% CI)
Cu	101 ± 7	89 ± 1	98 ± 1.4	100 ± 2	98.6 ± 5.0
Pb	160 ± 2	145 ± 6	145 ± 7	146 ± 1	161 ± 17
Zn	427 ± 2	411 ± 3	405 ± 14	427 ± 5	438 ± 12
Cd	NA	3.5 ± 0.66	3.7 ± 0.9	NA	3.45 ± 0.22
Cr	82 ± 3	79 ± 2	85 ± 4	89 ± 1	135 ± 5
Ni	42 ± 1	36 ± 1	38 ± 4	44 ± 2	44.1 ± 3.0

NA - Not Available

Table 4
Results of Analysis of NIST Standard Reference Material 2710
"Montana Soil (Highly Elevated Trace Element Concentrations)" Using Method 3050B
(µg/g ± SD)

Element	Atm. Pressure Microwave Assisted Method with Power Control	Atm. Pressure Microwave Assisted Method with Temperature Control (gas-bulb)	Atm. Pressure Microwave Assisted Method with Temperature Control (IR-sensor)	Hot-Plate	NIST Leachable Concentrations Using Method 3050	NIST Certified Values for Total Digestion (µg/g ±95% CI)
Cu	2640 ± 60	2790 ± 41	2480 ± 33	2910 ± 59	2700	2950 ± 130
Pb	5640 ± 117	5430 ± 72	5170 ± 34	5720 ± 280	5100	5532 ± 80
Zn	6410 ± 74	5810 ± 34	6130 ± 27	6230 ± 115	5900	6952 ± 91
Cd	NA	20.3 ± 1.4	20.2 ± 0.4	NA	20	21.8 ± 0.2
Cr	20 ± 1.6	19 ± 2	18 ± 2.4	23 ± 0.5	19	39*
Ni	7.8 ± 0.29	10 ± 1	9.1 ± 1.1	7 ± 0.44	10.1	14.3 ± 1.0

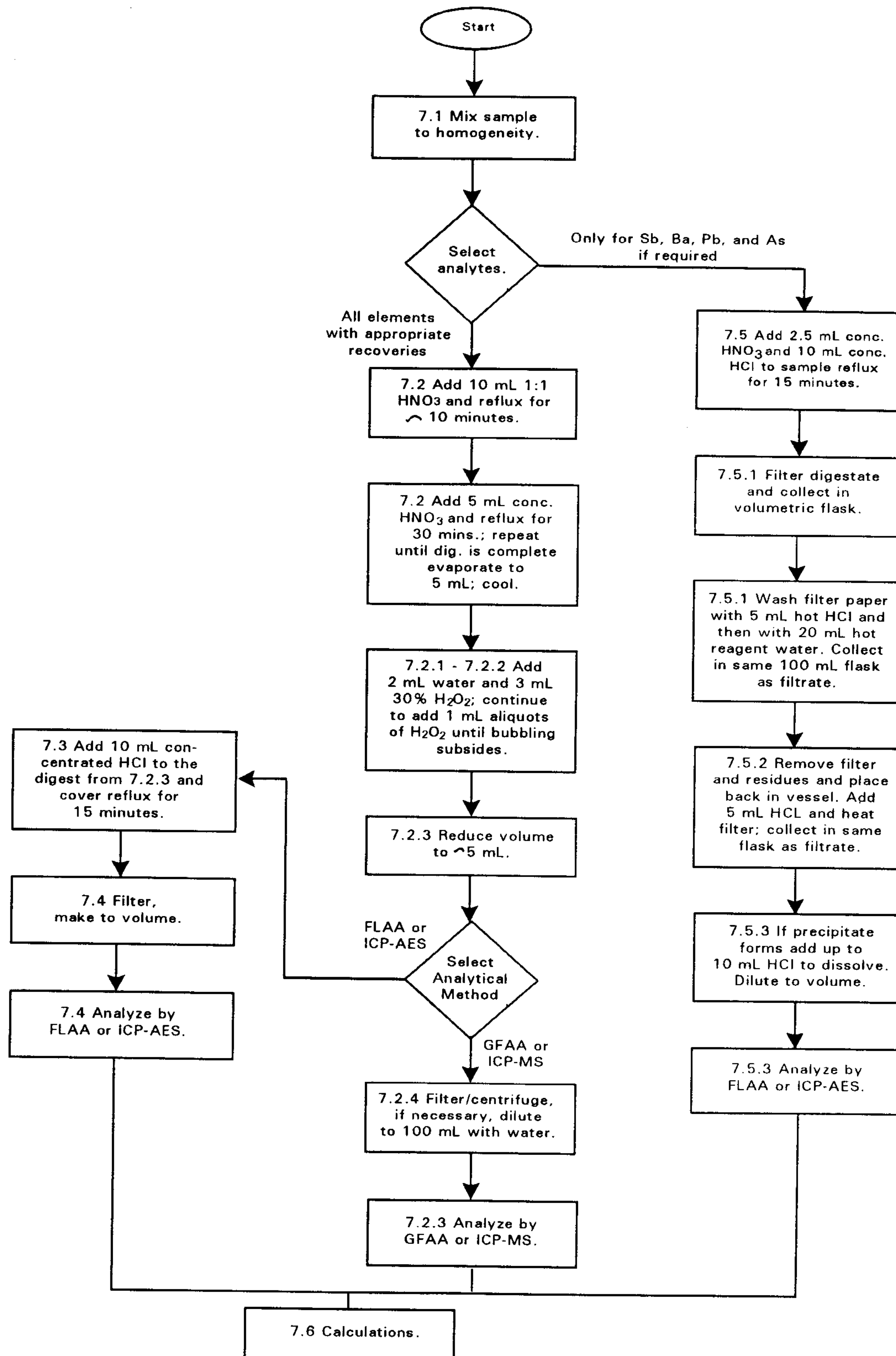
NA - Not Available * Non-certified values, for information only.

Table 5
Results of Analysis of NIST Standard Reference Material 2711
"Montana Soil (Moderately Elevated Trace Element Concentrations)" Using Method 3050B
(µg/g ± SD)

Element	Atm. Pressure Microwave Assisted Method with Power Control	Atm. Pressure Microwave Assisted Method with Temperature Control (gas-bulb)	Atm. Pressure Microwave Assisted Method with Temperature Control (IR-sensor)	Hot-Plate	NIST Leachable Concentrations Using Method 3050	NIST Certified Values for Total Digestion (µg/g ±95% CI)
Cu	107 ± 4.6	98 ± 5	98 ± 3.8	111 ± 6.4	100	114 ± 2
Pb	1240 ± 68	1130 ± 20	1120 ± 29	1240 ± 38	1100	1162 ± 31
Zn	330 ± 17	312 ± 2	307 ± 12	340 ± 13	310	350.4 ± 4.8
Cd	NA	39.6 ± 3.9	40.9 ± 1.9	NA	40	41.7 ± 0.25
Cr	22 ± 0.35	21 ± 1	15 ± 1.1	23 ± 0.9	20	47*
Ni	15 ± 0.2	17 ± 2	15 ± 1.6	16 ± 0.4	16	20.6 ± 1.1

NA - Not Available
* Non-certified values, for information only.

METHOD 3050B
ACID DIGESTION OF SEDIMENTS, SLUDGES, AND SOILS



METHOD 3052

MICROWAVE ASSISTED ACID DIGESTION OF SILICEOUS AND ORGANICALLY BASED MATRICES

1.0 SCOPE AND APPLICATION

1.1 This method is applicable to the microwave assisted acid digestion of siliceous matrices, and organic matrices and other complex matrices. If a total decomposition analysis (relative to the target analyte list) is required, the following matrices can be digested: ashes, biological tissues, oils, oil contaminated soils, sediments, sludges, and soils. This method is applicable for the following elements:

Aluminum	Cadmium	Iron	Molybdenum	Sodium
Antimony	Calcium	Lead	Nickel	Strontium
Arsenic	Chromium	Magnesium	Potassium	Thallium
Boron	Cobalt	Manganese	Selenium	Vanadium
Barium	Copper	Mercury	Silver	Zinc
Beryllium				

Other elements and matrices may be analyzed by this method if performance is demonstrated for the analyte of interest, in the matrices of interest, at the concentration levels of interest (see Sec. 8.0).

Note: This technique is not appropriate for regulatory applications that require the use of leachate preparations (i.e., Method 3050, Method 3051, Method 1311, Method 1312, Method 1310, Method 1320, Method 1330, Method 3031, Method 3040). This method is appropriate for those applications requiring a total decomposition for research purposes (i.e., geological studies, mass balances, analysis of Standard Reference Materials) or in response to a regulation that requires total sample decomposition.

1.2 This method is provided as a rapid multi-element, microwave assisted acid digestion prior to analysis protocol so that decisions can be made about the site or material. Digests and alternative procedures produced by the method are suitable for analysis by flame atomic absorption spectrometry (FLAA), cold vapor atomic absorption spectrometry (CVAA), graphite furnace atomic absorption spectrometry (GFAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and other analytical elemental analysis techniques where applicable. Due to the rapid advances in microwave technology, consult your manufacturer's recommended instructions for guidance on their microwave digestion system and refer to this manual’s "Disclaimer" when conducting analyses using Method 3052.

1.3 The goal of this method is total sample decomposition and with judicious choice of acid combinations this is achievable for most matrices (see Sec. 3.2). Selection of reagents which give the highest recoveries for the target analytes is considered the optimum method condition.

2.0 SUMMARY OF METHOD

2.1 A representative sample of up to 0.5 g is digested in 9 mL of concentrated nitric acid and usually 3 mL hydrofluoric acid for 15 minutes using microwave heating with a suitable laboratory microwave system. The method has several additional alternative acid and reagent combinations including hydrochloric acid and hydrogen peroxide. The method has provisions for scaling up the sample size to a maximum of 1.0 g. The sample and acid are placed in suitably inert polymeric microwave vessels. The vessel is sealed and heated in the microwave system. The temperature profile is specified to permit specific reactions and incorporates reaching 180 ± 5 ^oC in approximately less than 5.5 minutes and remaining at 180 ± 5 ^oC for 9.5 minutes for the completion of specific reactions (Ref. 1, 2, 3, 4). After cooling, the vessel contents may be filtered, centrifuged, or allowed to settle and then decanted, diluted to volume, and analyzed by the appropriate SW-846 method.

3.0 INTERFERENCES

3.1 Gaseous digestion reaction products, very reactive, or volatile materials that may create high pressures when heated and may cause venting of the vessels with potential loss of sample and analytes. The complete decomposition of either carbonates, or carbon based samples, may cause enough pressure to vent the vessel if the sample size is greater than 0.25 g. Variations of the method due to very reactive materials are specifically addressed in sections 7.3.4 and 7.3.6.1.

3.2 Most samples will be totally dissolved by this method with judicious choice of the acid combinations. A few refractory sample matrix compounds, such as TiO_2, alumina, and other oxides may not be totally dissolved and in some cases may sequester target analyte elements.

3.3 The use of several digestion reagents that are necessary to either completely decompose the matrix or to stabilize specific elements may limit the use of specific analytical instrumentation methods. Hydrochloric acid is known to interfere with some instrumental analysis methods such as flame atomic absorption (FLAA) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The presence of hydrochloric acid may be problematic for graphite furnace atomic absorption (GFAA) and inductively coupled plasma mass spectrometry (ICP-MS). Hydrofluoric acid, which is capable of dissolving silicates, may require the removal of excess hydrofluoric acid or the use of specialized non-glass components during instrumental analysis. Method 3052 enables the analyst to select other decomposition reagents that may also cause problems with instrumental analyses necessitating matrix matching of standards to account for viscosity and chemical differences.

4.0 APPARATUS AND MATERIALS

4.1 Microwave apparatus requirements.

4.1.1 The temperature performance requirements necessitate the microwave decomposition system sense the temperature to within ± 2.5°C and automatically adjust the microwave field output power within 2 seconds of sensing. Temperature sensors should be accurate to ± 2°C (including the final reaction temperature of 180°C). Temperature feedback control provides the primary control performance mechanism for the method. Due to the flexibility in the reagents used to achieve total analysis, tempertuare feedback control is necessary for reproducible microwave heating.

Alternatively, for a specific set of reagent(s) combination(s), quantity, and specific vessel type, a calibration control mechanism can be developed similar to previous microwave methods (see Method 3051). Through calibration of the microwave power, vessel load and heat loss, the reaction temperature profile described in Section 7.3.6 can be reproduced. The calibration settings are specific for the number and type of vessel used and for the microwave system in addition to the variation in reagent combinations. Therefore no specific calibration settings are provided in this method. These settings may be developed by using temperature monitoring equipment for each specific set of equipment and reagent combination. They may only be used if not altered as previously described in other methods such as 3051 and 3015. In this circumstance, the microwave system provides programmable power which can be programmed to within ± 12 W of the required power. Typical systems provide a nominal 600 W to 1200 W of power (Ref. 1, 2, 5). Calibration control provides backward compatibility with older laboratory microwave systems without temperature monitoring or feedback control and with lower cost microwave systems for some repetitive analyses. Older lower pressure vessels may not be compatible.

4.1.2 The temperature measurement system should be periodically calibrated at an elevated temperature. Pour silicon oil (a high temperature oil into a beaker and adequately stirred to ensure a homogeneous temperature. Place the microwave temperature sensor and a calibrated external temperature measurement sensor into the beaker. Heat the beaker to a constant temperature of 180 ± 5°C. Measure the temperature with both sensors. If the measured temperatures vary by more than 1 - 2°C, the microwave temperature measurement system needs to be calibrated. Consult the microwave manufacturer's instructions about the specific temperature sensor calibration procedure.

> CAUTION: The use of microwave equipment with temperature feedback control is required to control the unfamiliar reactions of unique or untested reagent combinations of unknown samples. These tests may require additional vessel requirements such as increased pressure capabilities.

4.1.3 The microwave unit cavity is corrosion resistant and well ventilated. All electronics are protected against corrosion for safe operation.

> CAUTION: There are many safety and operational recommendations specific to the model and manufacturer of the microwave equipment used in individual laboratories. A listing of these specific suggestions is beyond the scope of this method and require the analyst to consult the specific equipment manual, manufacturer, and literature for proper and safe operation of the microwave equipment and vessels.

4.1.4 The method requires essentially microwave transparent and reagent resistant suitably inert polymeric materials (examples are PFA or TFM suitably inert polymeric polymers) to contain acids and samples. For higher pressure capabilities the vessel may be contained within layers of different microwave transparent materials for strength, durability, and safety. The vessels internal volume should be at least 45 mL, capable of withstanding pressures of at least 30 atm (30 bar or 435 psi), and capable of controlled pressure relief. These specifications are to provide an appropriate, safe, and durable reaction vessel of which there are many adequate designs by many suppliers.

CAUTION: The outer layers of vessels are frequently not as acid or reagent resistant as the liner material and must not be chemically degraded or physically damaged to retain the performance and safety required. Routine examination of the vessel materials may be required to ensure their safe use.

CAUTION: The second safety concern relates to the use of sealed containers without pressure relief devices. Temperature is the important variable controlling the reaction. Pressure is needed to attain elevated temperatures, but must be safely contained. However, many digestion vessels constructed from certain suitably inert polymerics may crack, burst, or explode in the unit under certain pressures. Only suitably inert polymeric (such as PFA or TFM and others) containers with pressure relief mechanisms or containers with suitably inert polymeric liners and pressure relief mechanisms are considered acceptable.

Users are therefore advised not to use domestic (kitchen) type microwave ovens or to use inappropriate sealed containers without pressure relief for microwave acid digestions by this method. Use of laboratory-grade microwave equipment is required to prevent safety hazards. For further details, consult Reference 3 and 6.

4.1.5 A rotating turntable is employed to insure homogeneous distribution of microwave radiation within most systems (Ref. 1). The speed of the turntable should be a minimum of 3 rpm.

CAUTION: Laboratories should not use domestic (kitchen) type microwave ovens for this method. There are several significant safety issues. First, when an acid such as nitric is used to effect sample digestion in microwave units in open vessel(s), or sealed vessels equipment, there is the potential for the acid gas vapor released to corrode the safety devices that prevent the microwave magnetron from shutting off when the door is opened. This can result in operator exposure to microwave energy. Use of a system with isolated and corrosion resistant safety devices prevents this from occurring.

4.2 Volumetric ware, volumetric flasks, and graduated cylinders, 50 and 100 mL capacity or equivalent.

4.3 Filter paper, qualitative or equivalent.

4.4 Filter funnel, polypropylene, polyethylene or equivalent.

4.5 Analytical balance, of appropriate capacity, with a ± 0.0001 g or appropriate precision for the weighing of the sample. Optionally, the vessel with sample and reagents may be weighed, with an appropriate precision balance, before and after microwave processing to evaluate the seal integrity in some vessel types.

5.0 REAGENTS

5.1 All reagents should be of appropriate purity or high purity (acids for example, should be sub-boiling distilled where possible) to minimize the blank levels due to elemental contamination. All references to water in the method refer to reagent water (Ref. 7). Other reagent grades may be used, provided it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is questionable, analyze the reagent to determine the level of impurities. The reagent blank must be less than the MDL in order to be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be prewashed with detergents, acids, and water. Plastic and glass containers are both suitable. See Chapter Three, Sec. 3.1.3 of this manual, for further information.

6.3 Refer to Chapter Three for the appropriate holding times and storage conditions.

7.0 PROCEDURE

7.1 Temperature control of closed vessel microwave instruments provides the main feedback control performance mechanism for the method. Control requires a temperature sensor in one or more vessels during the entire decomposition. The microwave decomposition system should sense the temperature to within ± 2.5 ^{o}C and permit adjustment of the microwave output power within 2 seconds.

7.2 All digestion vessels and volumetric ware must be carefully acid washed and rinsed with reagent water. When switching between high concentration samples and low concentration samples, all digestion vessels (fluoropolymer liners only) should be cleaned by leaching with hot (1:1) hydrochloric acid (greater than 80°C, but less than boiling) for a minimum of two hours followed with hot (1:1) nitric acid (greater than 80°C, but less than boiling) for a minimum of two hours and rinsed with reagent water and dried in a clean environment. This cleaning procedure should also be used whenever the prior use of the digestion vessels is unknown or cross contamination from vessels is suspected. Polymeric or glass volumetric ware (not used with HF) and storage containers should be cleaned by leaching with more dilute acids (approximately 10% V/V) appropriate for the specific plastics used and then rinsed with reagent water and dried in a clean environment. To avoid precipitation of silver, ensure that all HCl has been rinsed from the vessels.

7.3 Sample Digestion

7.3.1 Weigh a well-mixed sample to the nearest 0.001 g into an appropriate vessel equipped with a pressure relief mechanism. For soils, ash, sediments, sludges, and siliceous wastes, initially use no more than 0.5 g. For oil or oil contaminated soils, initially use no more than 0.25 g.

7.3.2 Add 9 ± 0.1 mL concentrated nitric acid and 3 ± 0.1 mL concentrated hydrofluoric acid to the vessel in a fume hood. If the approximate silicon dioxide content of the sample is known, the quantity of hydrofluoric acid may be varied from 0 to 5 mL for stoichiometric reasons. Samples with higher concentrations of silicon dioxide (> 70%) may require higher concentrations of hydrofluoric acid (>3 mL HF). Alternatively samples with lower concentrations of silicon dioxide (< 10% to 0%) may require much less hydrofluoric acid (0.5 mL to 0 mL). Examples are presented in Table 1, 2, 3, and 6. Acid digestion reagent combinations used in the analysis of several matrices, listed in Table 7, provide guidance for the development of new matrix decomposition procedures.

7.3.3 The addition of other reagents with the original acids prior to digestion may permit more complete oxidation of organic sample constituents, address specific decomposition chemistry requirements, or address specific elemental stability and solubility problems.

The addition of 2 ± 2 mL concentrated hydrochloric acid to the nitric and hydrofluoric acids is appropriate for the stabilization of Ag, Ba, and Sb and high concentrations of Fe and Al in solution. The amount of HCl needed will vary depending on the matrix and the concentration of the analytes. The addition of hydrochloric acid may; however, limit the techniques or increase the difficulties of analysis. Examples are presented in Table 4.

The addition of hydrogen peroxide (30%) in small or catalytic quantities (such as 0.1 to 2 mL) may aid in the complete oxidation of organic matter.

The addition of water (double deionized) may (0 to 5 mL) improve the solubility of minerals and prevent temperature spikes due to exothermic reactions.

NOTE: Supporting documentation for the chemistry of this method has been prepared in chapters 2 and 3 of reference 3. It provides additional guidance and documentation of appropriate reagent, matrix and analyte combinations that can be employed in this method.

CAUTION: Only one acid mixture or quantity may be used in a single batch in the microwave to insure consistent reaction conditions between all vessels and monitored conditions. This limitation is due to the current practice of monitoring a representative vessel and applying a uniform microwave field to reproduce these reaction conditions within a group of vessels being simultaneously heated.

CAUTION: Toxic nitrogen oxide(s), hydrogen fluoride, and toxic chlorine (from the addition of hydrochloric acid) fumes are usually produced during digestion. Therefore, all steps involving open or the opening of microwave vessels must be performed in a properly operating fume ventilation system.

CAUTION: The analyst should wear protective gloves and face protection and must not at any time permit a solution containing hydrofluoric acid to come in contact with skin or lungs.

CAUTION: The addition of hydrochloric acid must be from concentrated hydrochloric acid and not from a premixed combination of acids as a buildup of toxic chlorine and possibly other gases will result from a premixed acid solution. This will over pressurize the vessel due to the release of these gases from solution upon heating. The gas effect is greatly lessened by following this suggestion.

CAUTION: When digesting samples containing volatile or easily oxidized organic compounds, initially weigh no more than 0.10 g and observe the reaction before capping the vessel. If a vigorous reaction occurs, allow the reaction to cease before capping the vessel. If no appreciable reaction occurs, a sample weight up to 0.25 g can be used.

CAUTION: The addition of hydrogen peroxide should only be done when the reactive components of the sample are known. Hydrogen peroxide may react rapidly and violently on easily oxidizable materials and should not be added if the sample may contain large quantities of easily oxidizable organic constituents.

7.3.4 The analyst should be aware of the potential for a vigorous reaction. If a vigorous reaction occurs upon the initial addition of reagent or the sample is suspected of containing easily oxidizable materials, allow the sample to predigest in the uncapped digestion vessel. Heat may be added in this step for safety considerations (for example the rapid release of carbon dioxide from carbonates, easily oxidized organic matter, etc.). Once the initial reaction has ceased, the sample may continue through the digestion procedure.

7.3.5 Seal the vessel according to the manufacturer's directions. Properly place the vessel in the microwave system according to the manufacturer's recommended specifications and connect appropriate temperature and pressure sensors to vessels according to manufacturer’s specifications.

7.3.6 This method is a performance based method, designed to achieve or approach total decomposition of the sample through achieving specific reaction conditions. The temperature of each sample should rise to 180 ± 5 ^{o}C in approximately 5.5 minutes and remain at 180 ± 5 ^{o}C for 9.5 minutes. The temperature-time and pressure-time profile are given for a standard soil sample in Figure 1. The number of samples simultaneously digested is dependent on the analyst. The number may range from 1 to the maximum number of vessels that the microwave units magnetron can heat according to the manufacturer’s or literature specifications (the number will depend on the power of the unit, the quantity and combination of reagents, and the heat loss from the vessels).

The pressure should peak between 5 and 15 minutes for most samples (Ref. 2, 3, 5). If the pressure exceeds the pressure limits of the vessel, the pressure will be reduced by the relief mechanism of the vessel.

The total decomposition of some components of a matrix may require or the reaction kinetics are dramatically improved with higher reaction temperatures. If microwave digestion systems and/or vessels are capable of achieving higher temperatures and pressures, the minimum digestion time of 9.5 minutes at a temperature of at least 180 ± 5°C is an appropriate

alternative. This change will permit the use of pressure systems if the analysis verifies that 180°C is the minimum temperature maintained by these control systems.

7.3.6.1 For reactive substances, the heating profile may be altered for safety purposes. The decomposition is primarily controlled by maintaining the reagents at 180 ± 5°C for 9.5 minutes, therefore the time it takes to heat the samples to 180 ± 5°C is not critical. The samples may be heated at a slower rate to prevent potential uncontrollable exothermic reactions. The time to reach 180 ± 5 ºC may be increased to 10 minutes provided that 180 ± 5 ºC is subsequently maintained for 9.5 minutes. Decomposition profiles are presented in Figures 1 and 2. The extreme difference in pressure is due to the gaseous digestion products.

7.3.6.2 Calibration control is applicable in reproducing this method provided the power in watts versus time parameters are determined to reproduce the specifications listed in 7.3.6. The calibration settings will be specific to the quantity and combination of reagents, quantity of vessels, and heat loss characteristics of the vessels (Ref 1). If calibration control is being used, any vessels containing acids for analytical blank purposes are counted as sample vessels and when fewer than the recommended number of samples are to be digested, the remaining vessels should be filled with the same acid mixture to achieve the full complement of vessels. This provides an energy balance, since the microwave power absorbed is proportional to the total absorbed mass in the cavity (Ref. 1). Irradiate each group of vessels using the predetermined calibration settings. (Different vessel types should not be mixed).

7.3.6.3 Pressure control for a specific matrix is applicable if instrument conditions are established using temperature control. Because each matrix will have a different reaction profile, performance using temperature control must be developed for every specific matrix type prior to use of the pressure control system.

7.3.7 At the end of the microwave program, allow the vessels to cool for a minimum of 5 minutes before removing them from the microwave system. When the vessels have cooled to near room temperature, determine if the microwave vessels have maintained a seal throughout the digestion. Due to the wide variability of vessel designs, a single procedure is not appropriate. For vessels that are sealed as discrete separate entities, the vessel weight may be taken before and after digestion to evaluate seal integrity. If the weight loss of sample exceeds 1% of the weight of the sample and reagents, then the sample is considered compromised. For vessels with burst disks, a careful visual inspection of the disk may identify compromised vessels. For vessels with resealing pressure relief mechanisms, an auditory or sometimes a physical sign indicates a vessel has vented.

7.3.8 Complete the preparation of the sample by carefully uncapping and venting each vessel in a fume hood. Vent the vessels using the procedure recommended by the vessel manufacturer. Transfer the sample to an acid-cleaned bottle. If the digested sample contains particulates which may clog nebulizers or interfere with injection of the sample into the instrument, the sample may be centrifuged, allowed to settle, or filtered.

7.3.8.1 Centrifugation: Centrifugation at 2,000 - 3,000 rpm for 10 minutes is usually sufficient to clear the supernatant.

7.3.8.2 Settling: If undissolved material remains such as TiO_2, or other refractory oxides, allow the sample to stand until the supernatant is clear. Allowing a sample to stand overnight will usually accomplish this. If it does not, centrifuge or filter the sample.

7.3.8.3 Filtering: If necessary, the filtering apparatus must be thoroughly cleaned and prerinsed with dilute (approximately 10% V/V) nitric acid. Filter the sample through qualitative filter paper into a second acid-cleaned container.

7.3.9 If the hydrofluoric acid concentration is a consideration in the analysis technique such as with ICP methods, boric acid may be added to permit the complexation of fluoride to protect the quartz plasma torch. The amount of acid added may be varied, depending on the equipment and the analysis procedure. If this option is used, alterations in the measurement procedure to adjust for the boric acid and any bias it may cause are necessary. This addition will prevent the measurement of boron as one of the elemental constituents in the sample. Alternatively, a hydrofluoric acid resistant ICP torch may be used and the addition of boric acid would be unnecessary for this analytical configuration. All major manufacturers have hydrofluoric resistant components available for the analysis of solutions containing hydrofluoric acid.

CAUTION: The traditional use of concentrated solutions of boric acid can cause problems by turning the digestion solution cloudy or result in a high salt content solution interfering with some analysis techniques. Dilute solutions of boric acid or other methods of neutralization or reagent elimination are appropriate to avoid problems with HF and the glass sample introduction devices of analytical instrumentation. Gentle heating often serves to clear cloudy solutions. Matrix matching of samples and standards will eliminate viscosity differences.

7.3.10 The removal or reduction of the quantity of the hydrochloric and hydrofluoric acids prior to analysis may be desirable. The chemistry and volatility of the analytes of interest should be considered and evaluated when using this alternative. Evaporation to near dryness in a controlled environment with controlled pure gas and neutralizing and collection of exhaust interactions is an alternative where appropriate. This manipulation may be performed in the microwave system, if the system is capable of this function, or external to the microwave system in more common apparatus(s). This option must be tested and validated to determine analyte retention and loss and should be accompanied by equipment validation possibly using the standard addition method and standard reference materials. This alternative may be used to alter either the acid concentration and/or acid composition. Note: The final solution typically requires nitric acid to maintain appropriate sample solution acidity and stability of the elements. Commonly, a 2% (v/v) nitric acid concentration is desirable. Examples of analysis performed with and without removal of the hydrofluoric acid are presented in Table 5. Waste minimization techniques should be used to capture reagent

fumes. This procedure should be tested and validated in the apparatus and on standards before using on unknown samples.

7.3.11 Transfer or decant the sample into volumetric ware and dilute the digest to a known volume. The digest is now ready for analysis for elements of interest using appropriate elemental analysis techniques and/or SW-846 methods.

7.3.12 Sample size may be scaled-up from 0.1, 0.25, or 0.5 g to 1.0 g through a series of 0.2g sample size increments. Scale-up can produce different reaction conditions and/or produce increasing gaseous reaction products. Increases in sample size may not require alteration of the acid quantity or combination, but other reagents may be added to permit a more complete decomposition and oxidation of organic and other sample constituents where necessary (such as increasing the HF for the complete destruction of silicates). Each step of the scale-up must demonstrate safe operation before continuing.

7.4 Calculations: The concentrations determined are to be reported on the basis of the actual weight of the original sample.

7.5 Calibration of Microwave Equipment

NOTE: If the microwave unit uses temperature feedback control to follow performance specifications of the method, then the calibration procedure will not be necessary.

7.5.1 Calibration is the normalization and reproduction of a microwave field strength to permit reagent and energy coupling in a predictable and reproducible manner. It balances reagent heating and heat loss from the vessels and is equipment dependent due to the heat retention and loss characteristics of the specific vessel. Available power is evaluated to permit the microwave field output in watts to be transferred from one microwave system to another.

Use of calibration to control this reaction requires balancing output power, coupled energy, and heat loss to reproduce the temperature heating profile in section 7.3.6. The conditions for each acid mixture and each batch containing the same specified number of vessels must be determined individually. Only identical acid mixtures and vessel models and specified numbers of vessels may be used in a given batch.

7.5.2 For cavity type microwave equipment, this is accomplished by measuring the temperature rise in 1 kg of water exposed to microwave radiation for a fixed period of time. The analyst can relate power in watts to the partial power setting of the system. The calibration format required for laboratory microwave systems depends on the type of electronic system used by the manufacturer to provide partial microwave power. Few systems have an accurate and precise linear relationship between percent power settings and absorbed power. Where linear circuits have been utilized, the calibration curve can be determined by a three-point calibration method (7.5.4), otherwise, the analyst must use the multiple point calibration method (7.5.3).

7.5.3 The multiple point calibration involves the measurement of absorbed power over a large range of power settings. Typically, for a 600 W unit, the following power settings are measured; 100, 99, 98, 97, 95, 90, 80, 70, 60, 50, and 40% using the procedure described in section 7.5.5. This data is clustered about the customary working power ranges. Nonlinearity has been encountered at the upper end of the calibration. If the system's electronics are known to have nonlinear deviations in any region of proportional power control, it will be necessary to make a set of measurements that bracket the power to be used. The final calibration point should be at the partial power setting that will be used in the test. This setting should be checked periodically to evaluate the integrity of the calibration. If a significant change is detected (±10 W), then the entire calibration should be reevaluated.

7.5.4 The three-point calibration involves the measurement of absorbed power at three different power settings. Measure the power at 100% and 50% using the procedure described in section 7.5.5. From the 2-point line calculate the power setting corresponding to the required power in watts specified in the procedure. Measure the absorbed power at that partial power setting. If the measured absorbed power does not correspond to the specified power within ±10 W, use the multiple point calibration in 7.5.3. This point should also be used to periodically verify the integrity of the calibration.

7.5.5 Equilibrate a large volume of water to room temperature (23 ± 2 ^oC). One kg of reagent water is weighed (1,000.0 g ± 0.1 g) into a suitably inert polymeric beaker or a beaker made of some other material that does not significantly absorb microwave energy (glass absorbs microwave energy and is not recommended). The initial temperature of the water should be 23 ± 2 ^oC measured to ± 0.05 ^oC. The covered beaker is circulated continuously (in the normal sample path) through the microwave field for 2 minutes at the desired partial power setting with the system's exhaust fan on maximum (as it will be during normal operation). The beaker is removed and the water vigorously stirred. Use a magnetic stirring bar inserted immediately after microwave irradiation and record the maximum temperature within the first 30 seconds to ± 0.05 ^oC. Use a new sample for each additional measurement. If the water is reused, both the water and the beaker must have returned to 23 ± 2 ^oC. Three measurements at each power setting should be made.

The absorbed power is determined by the following relationship:

Equation 1 $$P = \frac{K \; Cp \; m \; \Delta T}{t}$$

Where:

P	=	the apparent power absorbed by the sample in watts (W, W = joule sec^{-1})
K	=	the conversion factor for thermochemical calories_sec^{-1} to watts (which equals 4.184)
Cp	=	the heat capacity, thermal capacity, or specific heat (cal g^{-1} $^oC^{-1}$) of water

m	=	the mass of the water sample in grams (g)
ΔT	=	the final temperature minus the initial temperature (^oC)
t	=	the time in seconds (s)

Using the experimental conditions of 2 minutes and 1 kg of distilled water (heat capacity at 25 ^oC is 0.9997 cal g^{-1} $^oC^{-1}$) the calibration equation simplifies to:

$$P = 34.86\ \Delta T$$

NOTE: Stable line voltage is necessary for accurate and reproducible calibration and operation. The line voltage should be within manufacturer's specification, and during measurement and operation should not vary by more than ±5 V. Electronic components in most microwave units are matched to the system's function and output. When any part of the high voltage circuit, power source, or control components in the system have been serviced or replaced, it will be necessary to recheck the system's calibration. If the power output has changed significantly (±10 W), then the entire calibration should be reevaluated.

8.0 QUALITY CONTROL

8.1 All quality control data must be maintained and available for reference or inspection for a period determined by all involved parties based on program or project requirements. This method is restricted to use by, or under supervision of, experienced analysts. Refer to the appropriate section of Chapter One for additional quality control guidance.

8.2 Duplicate samples should be processed on a routine basis. A duplicate sample is a sample brought through the whole sample preparation and analytical process. A duplicate sample should be processed with each analytical batch or every 20 samples, whichever is the greater number. A duplicate sample should be prepared for each matrix type (i.e., soil, sludge, etc.).

8.3 Spiked samples and/or standard reference materials should be included with each group of samples processed or every 20 samples, whichever is the greater number. A spiked sample should also be included whenever a new sample matrix is being analyzed.

8.4 Blank samples should be prepared using the same reagents and quantities used in sample preparation, placed in vessels of the same type, and processed with the samples.

9.0 METHOD PERFORMANCE

9.1 Precision: Precision data for Method 3052 are presented in the tables of this method. Tables 1 through 6 provide a summary of total elemental analysis.

9.2 The performance criteria are provided as an example in Figure 1. The temperature profile will be within ± 5 ^oC of the mean of the temperature profile, but the pressure curve will vary depending on the acid mixture and gaseous digestion products and the thermal insulating properties of the vessel. Figure 2 provides criteria for the digestion of an oil sample.

10.0 REFERENCES

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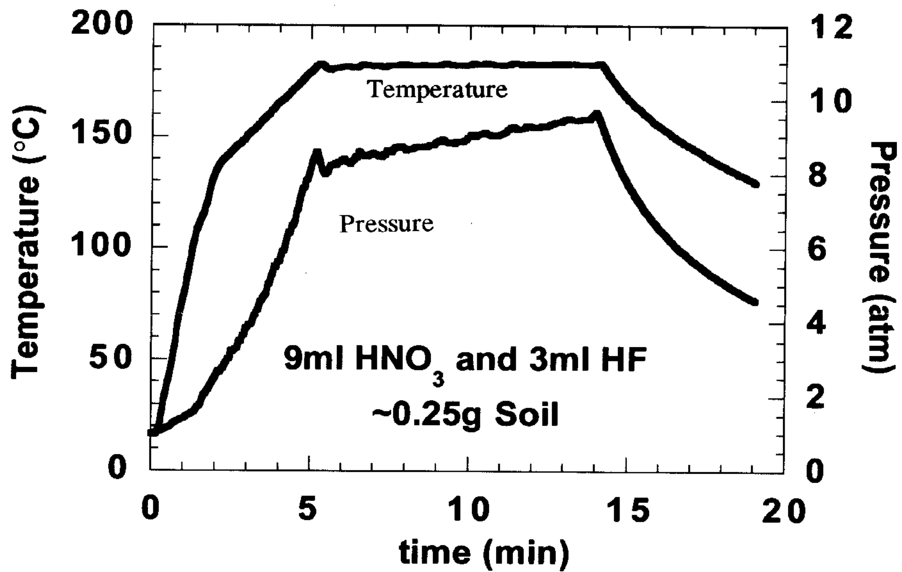


FIGURE 1. TYPICAL REACTION PROFILE FOR THE DIGESTION OF A SOIL (REF. 4 AND 8)

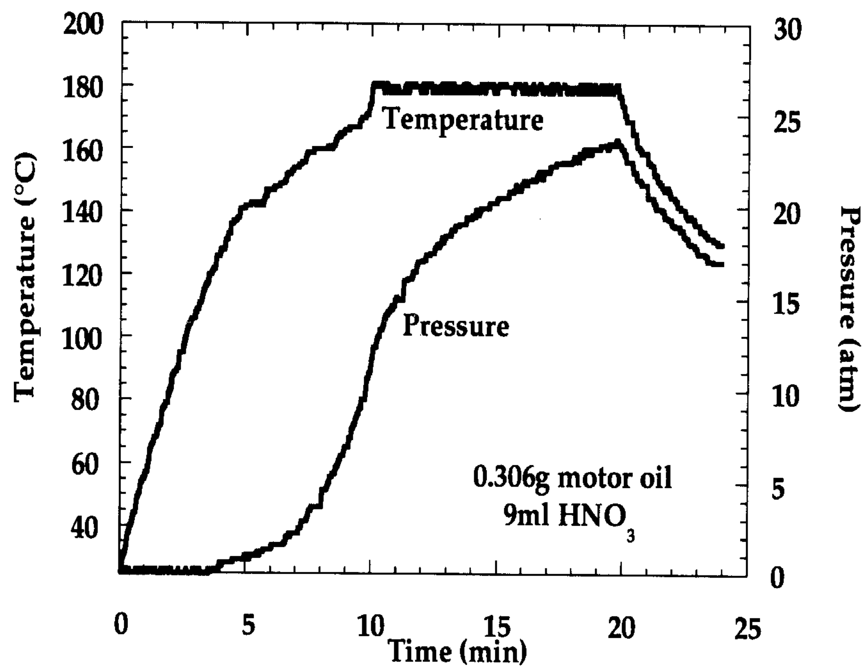


FIGURE 2. TYPICAL REACTION PROFILE FOR THE DIGESTION OF AN OIL (REF. 8)

TABLE 1
ANALYSIS OF NIST SRM 2704 (COMPILATION OF REFS. 2 AND 3)[a]
BUFFALO RIVER SEDIMENT

Element	Analyzed (µg/g)	Certified (µg/g)
Arsenic (n=4)	23.4 ± 2.6	23.4 ± 0.8
Cadmium (n=6)	3.5 ± 1.2	3.45 ± 0.22
Chromium (n=6)	132.9 ± 1.3	135 ± 5
Copper (n=6)	98.0 ± 4.2	98.6 ± 5.0
Lead (n=6)	155 ± 9.2	161 ± 17
Mercury (n=4)	1.49 ± 0.14	1.44 ± 0.07
Nickel (n=6)	43.6 ± 3.9	44.1 ± 3.0
Phosphorus (n=4)	1.016 ± 0.016 mg/g	0.998 ± 0.028 mg/g
Selenium (n=4)	1.13 ± 0.9	(1.1)
Sulfur (n=4)	3.56 ± 0.16	-----
Thallium (n=4)	1.15 ± 0.22	1.2 ± 0.2
Uranium (n=4)	2.97 ± 0.04	3.13 ± 0.13
Zinc (n=6)	441.9 ± 0.8	438 ± 12

[a] Digestion with 9 mL HNO_3 and 4 mL HF. Temperature and pressure conditions are as described in Section 7.3.6 of this method and similar to Figure 1. Data reported with 95% confidence intervals.

TABLE 2
ANALYSIS OF NIST SRM 2710 (REFS. 4 AND 3)[a]
MONTANA SOIL: HIGHLY ELEVATED TRACE ELEMENT CONCENTRATIONS (n=6)

Element	Analyzed (µg/g)	Certified (µg/g)
Antimony	39.3 ± 0.9 [b]	38.4 ± 3.0
Cadmium	21.9 ± 0.7 [a]	21.8 ± 0.2
Chromium	34.0 ± 3.2 [b]	(39)
Copper	2902 ± 83 [a]	2950 ± 130
Lead	5425 ± 251 [a]	5532 ± 80
Nickel	13.5 ± 1.0 [a]	14.3 ± 1.0
Silver	36.6 ± 0.5 [b]	35.3 ± 1.5
Zinc	7007 ± 111 [a]	6952 ± 91

[a] Digestion with either a. 9 mL HNO_3 and 4 mL HF or b. 9 mL HNO_3, 3 mL HF, & 2 mL HCl. Temperature and pressure conditions are as described in Sec. 7.3.6 of this method and similar to Figure 1. Data reported with 95% confidence intervals.

TABLE 3
NIST SRM 2711 (REFS. 4 AND 3)
MONTANA SOIL: MODERATELY ELEVATED TRACE ELEMENT CONCENTRATIONS (n=6)

Element	Analyzed (µg/g)	Certified (µg/g)
Cadmium	40.5 ± 1.0	41.70 ± 0.25
Chromium	45.5 ± 1.0	(47)
Copper	106.8 ± 3.4	114 ± 2
Lead	1161 ± 49	1162 ± 31
Nickel	19.6 ± 0.9	20.6 ± 1.1
Silver	4.3 ± 1.0	4.63 ± 0.39
Zinc	342 ± 9.4	350.4 ± 4.8

[a] Digestion with 9 mL HNO_3 and 4 mL HF. Temperature and pressure conditions are as described in Sec. 7.3.6 of this method and similar to Figure 1. Data reported with 95% confidence intervals.

TABLE 4
STABILIZATION AND RECOVERY OF ELEMENTS WITH HCl (REF. 3)[a] NIST SRM 2710
MONTANA SOIL: HIGHLY ELEVATED TRACE ELEMENT CONCENTRATIONS (n=6)

Element	HNO_3 & HF (µg/g)	HNO_3, HF & HCl (µg/g)	Certified (µg/g)
Antimony	33.1 ± 2.1	39.3 ± 0.9	38.4 ± 3.0
Silver	10.6 ± 4.5	36.6 ± 0.5	35.3 ± 1.5

[a] HNO_3 and HF - Digestion used 9 mL and 3 mL, respectively.
HNO_3, HF, and HCl - Digestion used 9 mL, 3 mL, and 2 mL respectively. Temperature and pressure conditions are as described in Sec. 7.3.6 of this method and similar to Figure 1. Data reported with 95% confidence intervals.

TABLE 5
FUMING OFF HYDROFLUORIC ACID WITH MICROWAVE EVAPORATION SYSTEM (REF 3)[a]
MONTANA SOIL: HIGHLY ELEVATED TRACE ELEMENT CONCENTRATIONS (n=4)

Element	Direct (µg/g)	Fumed (µg/g)	Certified (µg/g)
Antimony	39.3 ± 0.9	39.4 ± 0.9	38.4 ± 3.0
Cadmium	21.9 ± 0.7	23.3 ± 1.6	21.8 ± 0.2
Chromium	34.0 ± 3.2	32.4 ± 0.4	(39)
Copper	2902 ± 83	2870 ± 150	2950 ± 130
Lead	5425 ± 251	5502 ± 106	5532 ± 80
Nickel	13.5 ± 1.0	13.5 ± 0.8	14.3 ± 1.0
Silver	36.6 ± 0.5	38.9 ± 1.1	35.3 ± 1.5
Zinc	7007 ± 111	3992 ± 132	6952 ± 91

[a] Direct - Digestion used 9 mL HNO_3 and 3 mL HCl or 9 mL HNO_3, 3 mL HF, and 2 mL HCl
Fumed - Digestion used 9 mL HNO_3 and 3 mL HCl followed by the removal of the HF. Temperature and pressure conditions are as described in 7.3.6 of the method and similar to Figure 1. The digest solution was fumed in a microwave system under vacuum to ~1 mL and 3 mL HCl added. The digest solution was fumed to ~1 mL and 3 mL HNO_3 was added. The solution was fumed for a final step to ~1 mL and quantitatively transferred and diluted to final volume. Data reported with 95% confidence intervals.

TABLE 6
ANALYSIS OF NIST SRM 1084A (REF. 8) [a]
WEAR METALS IN OIL (100 ppm) (n=4)

Element	Analyzed (µg/g)	Certified (µg/g)
Chromium	98.1 ± 1.1	98.3 ± 0.8
Copper	1.2.4 ± 2.4	100.0 ± 1.9
Lead	99.2 ± 2.3	101.1 ± 1.3
Nickel	99.2 ± 2.4	99.7 ± 1.6
Silver	102.7 ± 2.2	101.4 ± 1.5

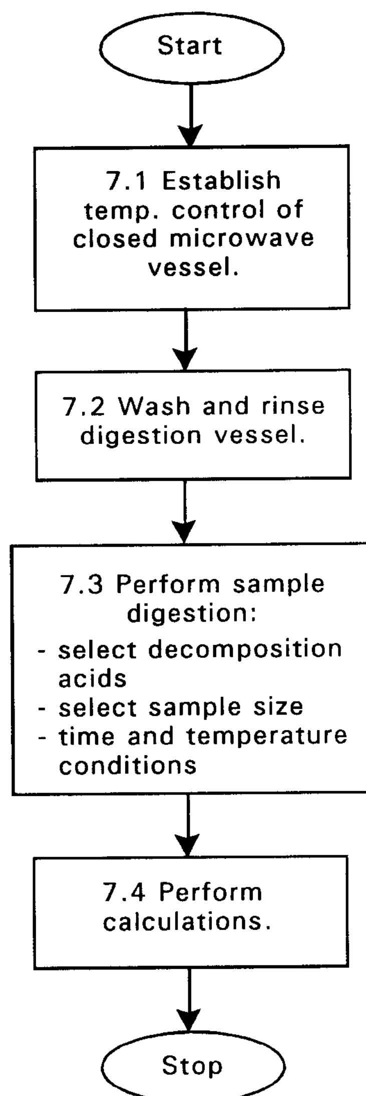
[a] Digestion with 9 mL HNO_3 and 0.5 mL HF. Temperature and pressure conditions are as described in Sec. 7.3.6 of this method and similar to Figure 2. Data reported with 95% confidence intervals.

TABLE 7
DIGESTION PARAMETERS USED IN THE ANALYSIS OF SEVERAL MATRICES
BY METHOD 3052

Matrix	HNO_3	HF	HCl
Soil			
NIST SRM 2710 Highly Contaminated Montana Soil	9 mL	3 mL	0-2*mL
NIST SRM 2711 Moderately Contaminated Montana Soil	9	3	0-2*
Sediment			
NIST SRM 2704 Buffalo River Sediment	9	3	0-2*
Biological			
NIST SRM 1566a Oyster Tissue	9	0	0
NIST SRM 1577a Bovine Liver	9	0	0
Botanical			
NIST SRM 1515 Apple Leaves	9	0	0
NIST SRM 1547 Peach Leaves	9	0	0
NIST SRM 1572 Citrus Leaves	9	0.5	0
Waste Oil			
NIST SRM 1084a Wear-Metals in Lubricating Oil	9	0.5	0-2*

* HCl is added to stabilize elements such as Ag and Sb when they are analyzed.

METHOD 3052
MICROWAVE ASSISTED ACID DIGESTION OF SILICEOUS AND ORGANICALLY BASED MATRICES



METHOD 3031

ACID DIGESTION OF OILS FOR METALS ANALYSIS BY ATOMIC ABSORPTION OR ICP SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method is an acid digestion procedure for analysis of oils, oil sludges, tars, waxes, paints, paint sludges and other viscous petroleum products for the sixteen toxic elements listed below:

Antimony	Arsenic	Barium	Beryllium
Cadmium	Chromium	Cobalt	Copper
Lead	Molybdenum	Nickel	Selenium
Silver	Thallium	Vanadium	Zinc

Other elements and matrices may be analyzed by this method if performance is demonstrated for the analytes of interest, in the matrix of interest, at the concentration levels of interest (see Section 8.0). The resulting digestate can be analyzed by either flame atomic absorption spectrometry (FLAA), graphite furnace atomic absorption spectrometry (GFAA), or inductively coupled plasma atomic emission spectrometry (ICP-AES).

1.2 The large concentration of manganese present in the digestate of Method 3031 can interfere with the determination of low concentrations of arsenic which is important for the recycled oil regulations. As an optional step, manganese may be removed from the digestate by forming a manganese phosphate precipitate. The remaining liquid can be analyzed by either flame atomic absorption spectrometry (FLAA) or inductively coupled plasma (ICP-AES). Chlorides can be removed by the use of nitric acid for analysis by graphite furnace atomic absorption spectrometry (GFAA) for arsenic. These clean-up procedures may be applicable to other elements as can be demonstrated by appropriate procedures (Sec. 7.11).

2.0 SUMMARY OF METHOD

2.1 A representative 0.5 g sample is mixed with 0.5 g of finely ground potassium permanganate and then 1.0 mL of concentrated sulfuric acid is added while stirring. A strong exothermic reaction occurs. The sample is then treated with 2 mL concentrated nitric acid. 10 mL of concentrated HCl is added and the sample is heated until the reaction is complete and is then filtered. The filter is washed with hot concentrated HCl. The filter paper is transferred to a digestion flask, treated with 5 mL of concentrated hydrochloric acid. The sample is brought to volume and analyzed by ICP-AES or FLAA.

> **<u>WARNING</u>: THIS PROCEDURE SHOULD NOT BE ATTEMPTED BY INEXPERIENCED PERSONNEL. MANY OF THE REACTIONS ARE STRONGLY EXOTHERMIC AND CAN RESULT IN SPLATTERING OR IN THE GENERATION OF GASES. GLOVES, FACESHIELDS, AND LAB COATS MUST BE WORN WHEN WORKING WITH ACIDS. IT IS STRONGLY RECOMMENDED THAT THE ADDITION OF SULFURIC ACID BE PERFORMED BEHIND A GLASS SHIELD OR SASH.**

2.2 To remove the manganese, the digestate is neutralized with concentrated ammonium hydroxide. Water and ammonium phosphate are added and the digestate is stirred while a precipitate of manganese ammonium phosphate is formed. When the precipitation is complete,

the digestate is filtered. The ammonia is then boiled off. The sample is brought to volume and analyzed on either ICP-AES or FLAA. For GFAA analysis, the volume is reduced and allowed to cool. Concentrated HNO_3 is added and the solution is heated. When the reaction is complete, bring to volume and analyze by GFAA.

3.0 INTERFERENCES

3.1 Most grades of potassium permanganate have elemental impurities that will interfere with the analysis. It is important that the permanganate be checked for purity. Background correction setting on an ICP-AES that are appropriate to the digestates of other matrices will not be effective for the digestates of oils. Background correction settings must be chosen for this unique digestate. These digestates can have very high dissolved solids, which may necessitate the use of internal standards, dilutions, or method of standard addition. Manganese is a very strong emitter and has many analytical lines. Analytical wavelengths must be chosen with care to avoid or minimize spectral overlap. Inter-element correction for manganese can be used for those instruments with that capability.

3.2 Excess ammonium hydroxide will result in the solubilization of some manganese.

3.3 To ensure comparable viscosities and chemistries between samples and standards, all standards must be matrix matched to the respective digestates.

4.0 APPARATUS AND MATERIALS

4.1 Beakers - 250 mL, or equivalent.

4.2 Temperature sensing device, e.g. thermometer, thermistor, thermocouple, or equivalent, capable of measuring temperatures between 0 and 150°C.

4.3 Filter paper - Whatman No. 41, or equivalent.

4.4 Funnels - polypropylene, or equivalent.

4.5 Heating device, e.g. hot plate, heating block, microwave or equivalent.

4.6 Volumetric flasks, of suitable precision and accuracy.

4.7 Volumetric pipettes, of suitable precision and accuracy.

4.8 Stirring device, e.g. magnetic stirrer, glass rod or equivalent.

NOTE: All glassware should be acid washed.

5.0 REAGENTS

5.1 Reagent Water. Reagent water will be interference free. All references to water in the method refer to reagent water unless otherwise specified. Refer to Chapter One of SW-846 for a definition of reagent water.

5.2 Nitric acid, concentrated, reagent grade (conc. HNO_3). Acid should be analyzed to determine level of impurities. If method blank is < MDL, then the acid can be used.

5.3 Hydrochloric acid, concentrated, reagent grade (conc. HCl). Acid should be analyzed to determine level of impurities. If method blank is < MDL, then the acid can be used.

5.4 Sulfuric acid, concentrated, reagent grade (conc. H_2SO_4). Acid should be analyzed to determine level of impurities. If method blank is < MDL, then the acid can be used.

5.5 Potassium permanganate - Ultra-pure grade. Reagent should be analyzed to determine level of impurities. If method blank is < MDL, then the reagent can be used.

5.6 Organometallic standards - scandium and/or yttrium may be used as internal standards for most samples. Standards traceable to NIST Standard No. 1085, for wear metals in oil, may be used.

5.7 Base oil, analyte-free. Oil should be analyzed to determine level of impurities. If method blank is < MDL, then the reagent can be used.

5.8 Ammonium hydroxide, concentrated, reagent grade - Reagent should be analyzed to determine level of impurities. If method blank is < MDL, then the acid can be used.

5.9 Ammonium phosphate, reagent grade - Reagent should be analyzed to determine level of impurities. If method blank is < MDL, then the acid can be used.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples must have been collected using a sampling plan that addresses the considerations discussed in Chapter Nine of this manual.

6.2 All sample containers must be pre-washed with detergents, acids, and water. See Chapter Three, Section 3.1.3, for further information.

6.3 Samples should be digested as soon as possible after arrival. Digestates may be kept for a period of 180 days in the case of most metals. See holding time table (Chapter Three) for specific metals of interest.

7.0 PROCEDURES

7.1 Homogenize sample and then take a representative sample of 0.5 g (± 0.01 g) and place in a beaker. Larger or smaller sample sizes can be used if needed.

> NOTE: Alternatively, with appropriate oils, H_2SO_4 and H_2O_2 may be used in repetition, with adjustments for stoichiometry, to permit the decomposition and reduce the dissolved solids content of digestate materials. If using an alternative reagent combination, equivalent performance must be demonstrated.

7.2 Add 0.5 g of potassium permanganate powder. If larger sample sizes are used, increase the amount of potassium permanganate so that the ratio of oil to potassium permanganate is still 1:1. Mix the oil and permanganate thoroughly until homogeneous. Thick oils and tars that cannot be mixed should be heated to achieve mixing (the oil may react mildly). It is important to record the amount of potassium permanganate used for each sample if analysis is by ICP-AES and correction is to be made for the amount of manganese.

If more than 10% of the sample is aromatic material, such as xylene, then the reaction will be incomplete. If this is the case, increase the amount of potassium permanganate. If the sample is a mixture of oil and other non-organic materials, reduce the amount of potassium permanganate.

NOTE: All steps requiring the use of acids should be conducted under a fume hood by properly trained personnel using appropriate laboratory safety equipment. This should include face shields and latex gloves.

7.3 Add 1.0 mL of concentrated H_2SO_4, and stir with an appropriate stirring device. If larger sample sizes are used, increase the volume of sulfuric acid so that ratio of oil to sulfuric acid is 1 g to 2 mL. The H_2SO_4 can be added dropwise or all at once, depending on analytical needs. (Generally, dropwise is preferred when low reporting limits are needed.)

NOTE: To prevent a strong exothermic reaction, H_2SO_4 should be added dropwise to all samples unfamiliar to the analyst and to all samples that are known to be highly reactive.

The reaction can take several seconds to begin, but when it occurs it will be very quick, vigorous, and exothermic. Generally larger sample sizes will react faster than smaller. Likewise, lower average molecular weight materials will react faster than heavier. Do not be mislead by an initial lack of reactivity. A grey-white vapor will be ejected from the beaker (SO_3) and splattering and bubbling can occur. The beaker will become very hot. This step is complete when no more gases are given off and the sample should be a thick black lumpy paste. Allow the beaker to cool as needed.

NOTE: Care must be taken when working with very light organic materials, such as diesel fuels, as they may flash. Generally, the lower the average molecular weight of the material correlates to a greater danger of flashing. The danger of flashing is reduced by adding the sulfuric acid dropwise.

NOTE: If more than 10% of the sample is aromatic material, such as xylene, only a little grey-white vapor will form. this will reduce accuracy and complicate nebulization. If there is a significant amount of non-hydrocarbon material, a sputtering reaction will occur and black MnO_2 particulates will be given off. See Section 7.2.

7.4 Add 2 mL of concentrated HNO_3 and stir. This reaction will be slightly exothermic. If larger sample sizes are used, it is not always necessary to increase the volume of HNO_3 proportionately, depending on analytical needs. Some reddish-brown vapor (NO_2) may be given off. Allow the reaction to continue until complete, that is when the digestate no longer gives off fumes. Allow the beaker to cool as needed.

7.5 Add 10 mL of concentrated HCl and stir. If larger sample sizes are used, it is not always necessary to increase the volume of HCl proportionately, depending on analytical needs. This reaction will be slightly exothermic and gas formation and foaming will occur. Lighter oils will foam more than will heavier oils. If excess foaming occurs, add water to prevent sample loss. Allow the beaker to cool as needed.

7.6 Heat the beaker until there is no further gas evolution. (temperature should not exceed 150° C to prevent volatilization). There may be additional foaming or other milder reactions which may result in overflow from the beaker. If excess foaming occurs, either remove the beaker from the heating source until foaming subsides or add sufficient water to prevent overflow. The final digestate should be a clear yellow liquid with black or dark reddish-brown particulates.

7.7 Filter the digestate through Whatman 41 filter paper and collect filtrate in a volumetric flask or beaker.

7.8 Wash the digestion beaker and filter paper, while still in the funnel, with no more than 5 mL of hot HCl.

NOTE: The purpose of this next step is to recover antimony, barium, and silver that may not have been complete solubilized. If the sample is not being prepared for these analytes, the next step may be skipped.

7.9 (Optional) After having washed the filter paper, remove the filter and residue from the funnel and place in back in the beaker. Add 5 mL of conc. HCl and place the beaker back on the heating source until the filter paper dissolves (temperature should not exceed 150°C ± 5°C to prevent volatilization). Remove the beaker from the heating source and wash the cover and sides with reagent grade water and then filter the residue and collect the filtrate in the same flask or beaker as in Sections 7.6 and 7.7. Allow the filtrate to cool and quantitatively transfer to a volumetric flask. Bring to volume.

7.10 (Optional) If the filtrate is collected in a beaker, the filtrate can be heated again to drive off excess HCl. This can reduce matrix effects in sample introduction (temperature should not exceed 150°C ± 5°C to prevent volatilization). When sufficient HCl has been removed, remove the beaker from the heating source, allow to cool, and then transfer the contents to a volumetric flask and bring to volume. However, if too much HCl is removed, barium, silver and antimony can be lost.

7.11 Analyze the filtrate by either ICP-AES or FLAA. Depending on the final volume selected, the total solids in the digestate may be high enough to cause nebulization problems. Problems due to high dissolved solids may be corrected by 1) following optional Section 7.9, 2) using internal standards, 3) using Flow Injection Analysis, or 4) using other matrix correction procedures.

Manganese Removal Steps

NOTE: The purpose of these next steps is to remove the manganese in the digest by precipitating it as manganese ammonium phosphate under alkaline conditions. Elements that do not form insoluble phosphates, such as arsenic, are filtered out and can be analyzed at lower concentrations.

7.12 Take the digestate, or portion of digestate and reduce the volume to remove as much HCl as possible without going below 10 mL. Then add conc. NH_4OH until pH is 7 or greater. For most matrices, the digestate will change colors (often from yellow to brown) at pH 7. A mild exothermic reaction will occur immediately.

7.13 Add at least 2 g ammonium phosphate for each 1 g of potassium permanganate used in the digestion and stir. An excess of phosphate is needed for good analyte recovery. Then add enough water and mix to ensure maximum precipitation. A pink or yellow silky amorphous precipitate, manganese ammonium phosphate, will form. If too much NH_4OH is used some of the manganese ammonium phosphate can be solubilized. Stir until precipitation is complete. Some ammonium phosphate may remain unreacted at the bottom of the beaker.

7.14 Filter the digestate through Whatman 41 filter paper (or equivalent) and collect filtrate in a volumetric flask or beaker.

7.15 Heat the filtrate to volatilize the ammonia (temperature should not exceed 150°C ± 5°C to prevent volatilization). the volume of filtrate can be reduced by heating to no less than 10 mL. If too much water is removed any ammonium chloride formed will solidify. If this occurs, either add enough water to dissolve the solids or filter out the solids and wash the residue with deionized water. A third alternative is to use nitric acid to destroy the ammonium chloride by using the step in Section 7.17.

7.16 The filtrate can be analyzed by ICP-AES or FLAA. The chlorides in the digestate will prevent the analysis by GFAA.

7.17 To analyze the digestate by GFAA, reduce the volume as much as possible. Cool and add sufficient conc. HNO_3 to drive off all chlorides. Heat gently and a mild exothermic reaction will occur. When no more reddish-brown gas (NO_2) is given off, the reaction is complete and the digestate can be cooled and taken to volume. This liquid can be analyzed by ICP-AES, FLAA, or GFAA.

8.0 QUALITY CONTROL

8.1 All quality control measures described in Chapter One should be followed.

8.2 For each analytical batch of samples processed, method blanks should be carried throughout the entire sample-preparation and analytical process. The blank will be useful in determining if samples are being contaminated. Do not subtract measured blank values from sample results. Use blanks to determine the source of contamination and eliminate it.

NOTE: This blank MUST include an analyte-free oil or explosive reactions can occur.

8.3 Duplicate samples should be processed on a routine basis. A duplicate sample is a sample brought through the whole sample preparation and analytical process. Refer to Chapter One for the proper protocol.

8.4 Organometallic standard reference materials (SRMs) or laboratory control samples spiked with organo-metallic standards should be employed to determine accuracy. Recoveries of SRMs and/or spikes should be +/- 25% of their true values.

9.0 METHOD PERFORMANCE

Refer to Tables 1, 2, 3, and 4.

10.0 REFERENCES

1. HMU 800, Acid Digestion of Oils for Metals Analysis by FLAA or ICP Spectroscopy, Southern California Laboratories.

TABLE 1
PERFORMANCE DATA USING SRM 1085[a]

Element	True Value (ppm)	Mean Value (ppm)	Percent Recovery	Standard Deviation (ppm)
Silver	306	283	92	35
Chromium	296	295	100	14
Copper	295	291	99	11
Molybdenum	303	283	93	23
Nickel	303	261	86	8.6
Lead	297	297	100	17
Vanadium	292	393	135	12

[a] n = 5

TABLE 2
PERCENT RECOVERIES AND STANDARD DEVIATIONS[ab]

Analyte	Method of Analysis	True Value (ppm)	Mean Value (ppm)	Percent Recovery	Standard Deviation (ppm)
Silver	ICP-AES	306	302	98	22
Silver	FLAA	306	254	83	6.7
Chromium	ICP-AES	296	278	94	19
Chromium	FLAA	296	240	81	16
Copper	ICP-AES	295	301	102	24
Copper	FLAA	295	250	85	11
Molybdenum	ICP-AES	303	282	93	12
Nickel	ICP-AES	303	262	86	24
Nickel	FLAA	303	237	78	9.3
Lead	ICP-AES	297	246	83	17
Lead	FLAA	297	260	88	4.2
Vanadium	ICP-AES	292	292	100	14

[a] Procedures tested using NIST SRM 1085.
[b] n = 12

TABLE 3
MEAN MEASURED VALUES FOR OIL STANDARDS BY SIMULTANEOUS ICP-AES[a]

	Concentration (μg/g)					
Analyte	500	100	50	25	5.0	2.5
Silver	472	90.2	46.2	23.1	5.15 (1)[b]	2.3 (1)[b]
Arsenic	146	67.9	39.0	18.1	1.8 (1)[b]	<1
Barium	31.0	26.6	8.4	5.8	4.67	2.17
Beryllium	575	113	56.6	28.2	6.26	3.25
Cadmium	442	83.5	43.87	21.6	3.96	1.67
Cobalt	441	82.3	42.4	20.7	3.36	0.69
Chromium	487	95.2	50.5	27.6	10.1	7.09
Copper	566	114	55.6	25.5	3.11	0.50
Molybdenum	529	95.7	48.7	26.1	6.47	3.64
Nickel	458	86.4	46.4	25.1	5.19	4.80
Lead	360	62.0	30.3	16.1	3.34	3.05
Antimony	667[c]	84.3	68.3	42.3	20.4	7.22
Selenium	350	93.0	50.1	25.8	11.8	11.6
Thallium	NA	72.2	37.6	28.1	10.9	<1
Vanadium	512	98.2	49.8	27.6	13.6	7.88
Zinc	512	93.2	43.8	16.8	1.6	<1

[a] n = 8
[b] Numbers in parenthesis represent the number of "less than" values.
[c] The highest standard for antimony was 1000 μg/g.

NA = Not Analyzed

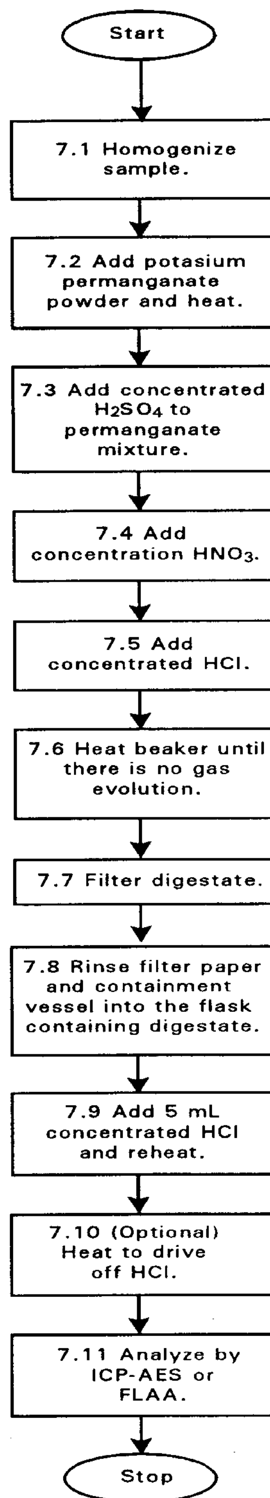
TABLE 4
STANDARD DEVIATIONS FOR OIL STANDARDS BY SIMULTANEOUS ICP-AES

Analyte	Concentration (µg/g) 500	100	50	25	5.0	2.5
Silver	14	3.6	1.1	4.1	6.3	0.46
Arsenic	3.1	4.1	1.7	1.9	1.1	[b]
Barium	0.88	9.2	4.0	5.9	0.30	0.18
Beryllium	3.4	1.5	1.5	0.41	0.35	0.46
Cadmium	2.1	1.7	0.73	0.66	0.53	0.26
Cobalt	2.1	1.8	0.69	1.3	0.24	0.30
Chromium	2.6	6.5	1.3	4.0	4.5	5.1
Copper	3.3	2.2	1.9	1.2	1.7	[b]
Molybdenum	3.2	1.6	0.62	1.0	0.69	0.36
Nickel	2.3	2.6	0.08	7.5	1.2	2.0
Lead	1.5	9.8	5.6	2.4	1.6	3.5
Antimony	34[c]	2.5	1.6	2.7	3.7	1.7
Selenium	5.7	5.4	6.8	8.0	6.4	4.3
Thallium	NA	8.5	13	18	8.2	[b]
Vanadium	3.8	4.4	0.84	7.2	11	8.3
Zinc	2.4	2.8	3.0	3.2	4.7	[b]

[a] n = 5
[b] The results were non-detects.
[c] The highest antimony standard was 1000 µg/g.

NA = Not Analyzed

METHOD 3031
ACID DIGESTION OF OILS FOR METALS
ANALYSIS BY ATOMIC ABSORPTION OR ICP SPECTROMETRY



Appendix B continued:

Methods Recommended by the Cannabis Science Task Force

Residual Solvents

- METHOD 8015D: Nonhalogenated Organics Using GC/FID
- METHOD 8260D: Volatile Organic Compounds By Gas Chromatography/Mass Spectrometry
- METHOD 3585: Waste Dilution For Volatile Organics
- METHOD 5021A: Volatile Organic Compounds In Various Sample Matrices Using Equilibrium Headspace Analysis

NONHALOGENATED ORGANICS USING GC/FID

1.0 SCOPE AND APPLICATION

1.1 This method may be used to determine the concentrations of various nonhalogenated volatile organic compounds and semivolatile organic compounds by gas chromatography. The following compounds have been determined quantitatively by this method, using the preparative techniques indicated.

Compound	CAS No.[a]	Appropriate Technique				
		Purge-and-Trap[b]	Head-space[e]	Direct Aqueous Injection	Azeo. Dist.[c]	Vacuum Dist.[d]
Acetone	67-64-1	pp / ht	x	x	x	x
Acetonitrile	75-05-8	pp	ne	x	x	ne
Acrolein	107-02-8	pp	ne	x	x	x
Acrylonitrile	107-13-1	pp	ne	x	x	x
Allyl alcohol	107-18-6	ht	ne	x	x	ne
t-Amyl alcohol (TAA)	75-85-4	ht	x	ne	ne	x
t-Amyl ethyl ether (TAEE)	919-94-8	x/ ht	x	ne	ne	x
t-Amyl methyl ether (TAME)	994-05-8	x/ ht	x	ne	ne	x
Benzene	71-43-2	x	x	ne	ne	x
t-Butyl alcohol (TBA)	75-65-0	ht	x	x	x	x
Crotonaldehyde	123-73-9	pp	ne	x	x	ne
Diethyl ether	60-29-7	x	ne	x	ne	ne
Diisopropyl ether (DIPE)	108-20-3	x/ ht	x	ne	ne	x
Ethanol	64-17-5	I	x	x	x	x
Ethyl acetate	141-78-6	I	x	x	x	ne
Ethyl Benzene	100-41-4	x	x	ne	ne	x
Ethylene oxide	75-21-8	I	ne	x	x	ne
Ethyl *tert*-butyl ether (ETBE)	637-92-3	x/ ht	x	ne	ne	x
Isopropyl alcohol (2-Propanol)	67-63-0	pp	x	x	x	ne
Methanol	67-56-1	I	x	x	x	ne
Methyl ethyl ketone (MEK, 2-Butanone)	78-93-3	pp	x	x	x	x
Methyl *tert*-butyl ether (MTBE)	1634-04-4	x/ ht	x	x	ne	x
N-Nitroso-di-*n*-butylamine	924-16-3	pp	ne	x	x	ne
Paraldehyde	123-63-7	pp	ne	x	x	ne
2-Pentanone	107-87-9	pp	x	x	x	ne
2-Picoline	109-06-8	pp	ne	x	x	ne
1-Propanol (*n*-Propyl alcohol)	71-23-8	pp	x	x	x	ne
Propionitrile	107-12-0	ht	ne	x	x	ne
Pyridine	110-86-1	I	ne	x	x	ne
Toluene	108-88-3	x	x	ne	ne	x

Compound	CAS No.[a]	Appropriate Technique				
		Purge-and-Trap[b]	Head-space[e]	Direct Aqueous Injection	Azeo. Dist.[c]	Vacuum Dist.[d]
o-Toluidine	95-53-4	I	ne	x	x	ne
o-Xylene	95-47-6	x	x	ne	ne	x
m-Xylene	108-38-3	x	x	ne	ne	x
p-Xylene	106-42-3	x	x	ne	ne	x

a Chemical Abstracts Service Registry Number
b Purge-and-Trap (Methods 5030 or 5035)
c Azeotropic distillation (Method 5031)
d Vacuum distillation (Method 5032)
e Headspace (Method 5021)
x Adequate response using this technique
ht Method analyte only when purged at 80 °C (high temperature purge)
I Inappropriate technique for this analyte
ne Not evaluated
pp Poor purging efficiency, resulting in higher quantitation limits. Use of an alternative sample preparative method is strongly recommended. May be amenable to purging at elevated temperature.

1.2 This method is applicable to the analysis of other analytes, including triethylamine and petroleum hydrocarbons. The petroleum hydrocarbons include gasoline range organics (GRO) and diesel range organics (DRO). The sample preparation techniques are shown in the table below.

Compound	CAS No.[a]	Appropriate Technique			
		Purge-and-Trap	Head-space	Direct Aqueous Injection	Solvent Extraction
Triethylamine	121-44-8	I	ne	x	I
Gasoline range organics (GRO)	--	x	x	x	I
Diesel range organics (DRO)	--	I	x	I	x

a Chemical Abstracts Service Registry Number
x Adequate response using this technique
I Inappropriate technique for this analyte
ne Not evaluated

1.2.1 This method has been applied to the analysis of triethylamine in water samples by direct aqueous injection onto a different GC column than is used for any other analytes. Descriptions of the GC column, temperature program, and performance data for triethylamine are provided in this method (see Secs. 6.2.5 and 11.2.6, and Table 8).

1.2.2 GRO corresponds to the range of alkanes from C_6 to C_{10} and covering a boiling point range of approximately 60°C - 170°C (Reference 6). DRO corresponds to the range of alkanes from C_{10} to C_{28} and covering a boiling point range of approximately 170°C -

430°C (Reference 6). The quantitative analyses of these fuel types are based on the procedures described in Sec. 11.11. The identification of specific fuel types may be complicated by environmental processes such as evaporation, biodegradation, or when more than one fuel type is present. Methods from other sources may be more appropriate for GRO and DRO, since these hydrocarbons are not regulated under RCRA. Consult State and local regulatory authorities for specific requirements.

1.2.3 This method may be applicable to classes of analytes and to fuel types and petroleum hydrocarbons other than those listed in Secs. 1.1 and 1.2. However, in order to be used for additional analytes, fuel types, or petroleum hydrocarbons, the analyst must demonstrate that the gas chromatographic conditions, including the GC column, are appropriate for the analytes of interest. The analyst must also perform the initial demonstration of proficiency described in Sec. 9.6 and Method 8000. Expansion of this method to other fuel types or petroleum hydrocarbons will also require that the boiling point range or carbon number range of the material be carefully defined and the quantitation approach be modified to match such ranges. Analysts are advised to consult authoritative sources, such as the American Petroleum Institute (API), for appropriate definitions of other fuel types or petroleum fractions.

NOTE: Mention of the analyses of other fuel types and petroleum fractions does *not* imply a regulatory requirement for such analyses, using this or any other method.

1.3 The method can also be used as a screening tool (for both volatile and semivolatile organics) to obtain semiquantitative data to prevent overloading the GC/MS system during quantitative analysis. This may be accomplished using a purge-and-trap method (e.g., Method 5030), an automated headspace method (e.g., Method 5021), direct aqueous injection, or by direct injection, if a solvent extraction method has been utilized for sample preparation. Single-point calibration is acceptable in this situation. Performance data are not provided for screening.

1.4 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly required in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.5 This method is restricted for use by, or under the supervision of, analysts experienced in the use of a gas chromatograph and skilled in the interpretation of gas chromatograms. In addition, if this method is used for the analysis of petroleum hydrocarbons, it is limited to analysts experienced in the interpretation of hydrocarbon data. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 This method provides gas chromatographic conditions for the detection of certain nonhalogenated volatile and semivolatile organic compounds.

2.2 Depending on the analytes of interest, samples may be introduced into the GC by a variety of techniques, including:

- Purge-and-trap (Methods 5030 or 5035)
- Equilibrium headspace (Method 5021)
- Direct injection of aqueous samples
- Injection of the concentrate from azeotropic distillation (Method 5031)
- Vacuum distillation (Method 5032)
- Following solvent extraction (Methods 3510, 3520, 3535, 3540, 3541, 3545, 3546, 3550, 3560, or other appropriate technique)

2.3 Groundwater or surface water samples generally must be analyzed in conjunction with Methods 5021, 5030, 5031, 5032, 3510, 3520, or other appropriate preparatory methods to obtain the necessary quantitation limits. Method 3535 (solid-phase extraction) may also be applicable to some of the target analytes, but has not been validated by EPA in conjunction with this determinative method.

2.4 Samples to be analyzed for diesel range organics may be prepared by an appropriate solvent extraction method.

2.5 Gasoline range organics may be introduced into the GC/FID by purge-and-trap (Methods 5030 and 5035), automated headspace (Method 5021), vacuum distillation (Method 5032), or other appropriate technique.

2.6 Triethylamine may be analyzed by direct injection of aqueous samples. This compound has not been found to be amenable to purge-and-trap techniques.

2.7 An appropriate column and temperature program are used in the gas chromatograph to separate the organic compounds. Detection is achieved by a flame ionization detector (FID).

2.8 The method allows the use of packed or capillary columns for the analysis and confirmation of the non-halogenated individual analytes. The GC columns and conditions listed have been demonstrated to provide separation of those target analytes. Other columns and conditions may be employed, provided that the analyst demonstrates adequate performance for the intended application.

2.9 The quantitative analyses of GRO and DRO are based on the definitions provided in Sec. 1.2.2 and the procedures described in Sec. 11.11.

2.10 Given the large number of components to be separated, fused-silica capillary columns are necessary for the analysis of petroleum hydrocarbons, including GRO and DRO, and are recommended for all other analytes. A capillary column is also necessary for the analysis of triethylamine.

3.0 DEFINITIONS

Refer to the SW-846 chapter of terms and acronyms for potentially applicable definitions.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be necessary. Refer to each method for specific guidance on quality control procedures and to Chapter Four for guidance on the cleaning of glassware.

4.2 When analyzing for volatile organics, samples can be contaminated by diffusion of volatile organics (particularly chlorofluorocarbons and methylene chloride) through the sample container septum during shipment and storage. A trip blank prepared from organic-free reagent water and carried through sampling and subsequent storage and handling will serve as a check on such contamination.

4.3 Contamination by carryover can occur whenever high-concentration and low-concentration samples are analyzed in sequence. To reduce the potential for carryover, the sample syringe or purging device must be rinsed out between samples with an appropriate solvent. Whenever an unusually concentrated sample is encountered, it should be followed by injection of a solvent blank to check for cross contamination.

4.3.1 Clean purging vessels with a detergent solution, rinse with distilled water, and then dry in a 105°C oven between analyses. Clean syringes or autosamplers by flushing all surfaces that contact samples using appropriate solvents.

4.3.2 All glassware must be scrupulously cleaned. Clean all glassware as soon as possible after use by rinsing with the last solvent used. This should be followed by detergent washing with hot water, and rinses with tap water and organic-free reagent water. Drain the glassware and dry it in an oven at 130°C for several hours or rinse it with methanol and drain. Store dry glassware in a clean environment.

4.4 The flame ionization detector (FID) is a non-selective detector. There is a potential for many non-target compounds present in samples to interfere with this analysis. There is also the potential for analytes to be resolved poorly, especially in samples that contain many analytes. The data user should consider this and may wish to alter the target analyte list accordingly.

5.0 SAFETY

There are no significant safety issues specific to this method. However, SW-846 methods do not purport to address all safety issues associated with their use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

6.1 Gas chromatograph - Analytical system complete with gas chromatograph suitable for solvent injections, direct aqueous injection, headspace, vacuum distillation sample introduction, or purge-and-trap sample introduction, and equipped with all necessary accessories, including detectors, column supplies, recorder, gases, and syringes. A data system for measuring peak heights and/or peak areas is recommended.

6.2 Recommended GC columns

The choice of GC column will depend on the analytes of interest, the expected concentrations, and the intended use of the results. The packed columns listed below are generally used for screening analyses. The capillary columns are necessary for petroleum hydrocarbon analyses and for triethylamine analyses and are recommended for all other analyses. Other columns and columns of other diameters may be employed if the analyst can demonstrate acceptable performance for the intended application.

6.2.1 Column 1 - 8-ft x 0.1-in. ID stainless steel or glass column, packed with 1% SP-1000 on Carbopak-B 60/80 mesh or equivalent.

6.2.2 Column 2 - 6-ft x 0.1-in. ID stainless steel or glass column, packed with *n*-octane on Porasil-C 100/120 mesh (Durapak) or equivalent.

6.2.3 Column 3 - 30-m x 0.53-mm ID fused-silica capillary column bonded with DB-Wax (or equivalent), 1-µm film thickness.

6.2.4 Column 4 - 30-m x 0.53-mm ID fused-silica capillary column chemically bonded with 5% methyl silicone (DB-5, SPB-5, RTx, or equivalent), 1.5-µm film thickness.

6.2.5 Column 5 - 30-m x 0.53-mm ID fused-silica capillary column bonded with HP Basic Wax (or equivalent), 1-µm film thickness. This column is used for triethylamine.

6.2.6 Wide-bore columns should be installed in 1/4-inch injectors, with deactivated liners designed specifically for use with these columns.

6.3 Detector - Flame ionization (FID)

6.4 Sample introduction and preparation apparatus

6.4.1 Refer to the 5000 series sample preparation methods for the appropriate apparatus for purge-and-trap, headspace, azeotropic distillation, and vacuum distillation analyses.

6.4.2 Samples may also be introduced into the GC via injection of solvent extracts or direct injection of aqueous samples.

6.5 Syringes

6.5.1 5-mL Luer-Lok glass hypodermic and 5-mL gas-tight syringe with shutoff valve, for volatile analytes.

6.5.2 Microsyringes - 10- and 25-µL with a 0.006-in. ID needle (Hamilton 702N or equivalent) and 100-µL.

6.6 Volumetric flasks, Class A - Appropriate sizes with ground-glass stoppers.

6.7 Analytical balance - 160-g capacity, capable of measuring to 0.0001 g.

7.0 REAGENTS AND STANDARDS

7.1 Reagent grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 Organic-free reagent water. All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

7.3 Methanol, CH_3OH - Pesticide quality or equivalent. Store away from other solvents.

7.4 Fuels, e.g., gasoline or diesel - Purchase from a commercial source. Low-boiling components in fuel evaporate quickly. If available, obtain fuel from the leaking tank on site.

7.5 Alkane standard - A standard containing a homologous series of *n*-alkanes for establishing retention times (e.g., C_{10}-C_{28} for diesel).

7.6 Stock standards - Stock solutions may be prepared from pure standard materials or purchased as certified solutions. When methanol is a target analyte or when using azeotropic

distillation for sample preparation, standards should not be prepared in methanol. Standards must be replaced after 6 months or sooner, if comparison with check standards indicates a problem.

7.7 Secondary dilution standards - Using stock standard solutions, prepare secondary dilution standards, as needed, that contain the compounds of interest, either singly or mixed together. The secondary dilution standards should be prepared at concentrations such that the aqueous calibration standards prepared in Sec. 7.8 will bracket the working range of the analytical system. Secondary dilution standards should be stored with minimal headspace for volatiles and should be checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards from them.

7.8 Calibration standards - Calibration standards at a minimum of five different concentrations are prepared in organic-free reagent water (for purge-and-trap, direct aqueous injection, azeotropic distillation, or vacuum distillation) or in methylene chloride (for solvent injection) from the secondary dilution of the stock standards. For headspace, the standards are prepared as directed in Method 5021. One of the standards should be at or below the concentration equivalent to the appropriate quantitation limit for the project. The remaining concentrations should correspond to the expected range of concentrations found in real samples or should define the working range of the GC. Each standard should contain each analyte to be determined by this method (e.g., some or all of the compounds listed in Sec. 1.1 may be included). In order to prepare accurate aqueous standard solutions, the following precautions must be observed:

7.8.1 Do not inject more than 20 µL of methanolic standards into 100 mL of water.

7.8.2 Use a 25-µL Hamilton 702N microsyringe or equivalent (variations in needle geometry will adversely affect the ability to deliver reproducible volumes of methanolic standards into water).

7.8.3 Rapidly inject the primary standard into the filled volumetric flask. Remove the needle as fast as possible after injection.

7.8.4 Mix diluted standards by inverting the flask three times only.

7.8.5 Fill the sample syringe from the standard solution contained in the expanded area of the flask (do not use any solution contained in the neck of the flask).

7.8.6 The negative pressure generated by pipettes makes them inappropriate for routine use in the transfer of spiked solutions. As such, use of pipettes to dilute or transfer aqueous standards should be avoided. When sample transfer is absolutely necessary, (such as in the performance of headspace sample preparation for water samples) only high quality, automatic pipettes should be used, and then with extreme care.

7.8.7 Aqueous standards used for purge-and-trap analyses (Method 5030) are not stable and should be discarded after 1 hour, unless held in sealed vials with zero headspace. If so stored, they may be held for up to 24 hours. Aqueous standards used for azeotropic distillation (Method 5031) may be stored for up to a month in polytetrafluoroethylene (PTFE)-sealed screw-cap bottles with minimal headspace, at 4°C, and protected from light.

7.8.8 Standards for direct aqueous injection of triethylamine are prepared by dissolving an appropriate weight of neat triethylamine in organic-free reagent water and diluting to volume in a volumetric flask.

7.9 Internal standards (if internal standard calibration is used) - To use this approach, the analyst must select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst must further demonstrate that the measurement of the internal standard is not affected by method or matrix interferences. Because of these limitations, no internal standard can be suggested that is applicable to all samples. The following internal standards are recommended when preparing samples by azeotropic distillation (Method 5031): 2-chloroacrylonitrile, hexafluoro-2-propanol, and hexafluoro-2-methyl-2-propanol.

7.10 Surrogate standards - Whenever possible, the analyst should monitor both the performance of the analytical system and the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard, and blank with one or two surrogate compounds which are not affected by method interferences.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 See the introductory material to this chapter, Organic Analytes, Sec. 4.1 and Method 5035A.

8.2 If the headspace technique is used, also see Method 5021.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One.

9.2 Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. Each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

9.3 Any method blanks, matrix spike samples, or replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.4 Refer to Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Methods 3500 and 5000. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

9.5 Quality control procedures necessary to evaluate the GC system operation are found in Method 8000, Sec. 9.0, and include evaluation of retention time windows, calibration verification and chromatographic analysis of samples.

9.6 Initial demonstration of proficiency - Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Methods 5000 and 8000 for information on how to accomplish this demonstration.

9.7 Sample quality control for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and detection limit). At a minimum, this includes the analysis of QC samples including a method blank, a matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

9.7.1 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

9.7.2 A laboratory control sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

9.7.3 See Method 8000, Sec. 9.0, for the details on carrying out sample quality control procedures for preparation and analysis.

9.8 Surrogate recoveries - The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 9.0, for information on evaluating surrogate data and developing and updating surrogate limits.

9.9 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Introduction/preparation methods

Various techniques are available for sample introduction. All internal standards, surrogates, and matrix spikes (when applicable) must be added to samples before introduction into the GC/FID system. Consult the applicable sample introduction method regarding when to add standards.

Other sample introduction techniques may be appropriate for specific applications and the techniques described here also may be appropriate for other matrices and analytes. Whatever technique is employed, *including* those specifically listed below, the analyst *must* demonstrate adequate performance for the analytes of interest. At a minimum, such a demonstration will encompass the initial demonstration of proficiency described in Sec. 9.6, using a clean reference matrix. Method 8000 describes procedures that may be used to develop performance criteria for such demonstrations as well as for matrix spike and laboratory control sample results.

11.1.1 Direct aqueous injection - This technique involves direct syringe injection of an aliquot of an aqueous sample into the GC injection port. This technique is applicable to the following groups of analytes in this method.

11.1.1.1 Volatile organics (includes GRO)

This technique may involve injection of an aqueous sample containing a very high concentration of analytes. Direct injection of aqueous samples has very limited application in the analysis of volatile organics. It is only appropriate for the determination of volatiles at the toxicity characteristic (TC) regulatory limits or at concentrations in excess of 10,000 µg/L. It may also be used in conjunction with the test for ignitability in aqueous samples (along with Methods 1010 and 1020) to determine if alcohol is present at > 24%.

11.1.1.2 Triethylamine in aqueous samples

Triethylamine may be determined by injecting a portion of an aqueous sample directly into the GC injection port. This technique has been demonstrated to be appropriate for samples containing low µg/L (ppb) concentrations of triethylamine.

11.1.2 Purge-and-trap of volatile organics (includes GRO)

This includes purge-and-trap for aqueous samples (Method 5030) and purge-and-trap for solid samples (Method 5035). Method 5035 also provides techniques for extraction of solid and oily waste samples by methanol (and other water-miscible solvents) with subsequent purge-and-trap from an aqueous matrix using Method 5030. Normally, purge-and-trap for aqueous samples is performed at ambient temperatures, while soil/solid samples utilize a 40°C purge to improve extraction efficiency. Heated purge may also be used to improve the purging of compounds with high solubilities in water, particularly alcohols associated with fuel oxygenates such as TBA and TAA. Occasionally, there may be a need to perform a heated purge for aqueous samples to lower detection limits; however, a 25-mL sample will often provide the sensitivity needed in most situations.

11.1.3 Azeotropic distillation

This technique exploits the ability of selected water-soluble organic compounds to form binary azeotropes with water during distillation. The organic compounds are removed from the bulk water sample and concentrated in a distillate, as described in Method 5031. An aliquot of the distillate is then injected into the GC/FID.

11.1.4 Vacuum distillation of volatile organics

This technique employs a vacuum distillation apparatus to introduce volatile organics from aqueous, solid, or tissue samples into the GC/FID system, as described in Method 5032.

11.1.5 Automated static headspace

This technique employs a device that collects the volatile organics from the headspace over a sample contained in a sealed vial and introduces them into the GC/FID system, as described in Method 5021.

11.1.6 Solvent injection

This technique involves the syringe injection of solvent extracts of aqueous samples prepared by Methods 3510, 3520, 3535, or other appropriate technique, or extracts of soil/solids prepared by Methods 3540, 3541, 3545, 3546, 3550, 3560, or other appropriate technique. It is applicable to many semivolatile organics, including DRO.

WARNING: Ultrasonic extraction (Method 3550) may not be as rigorous a method as the other extraction methods for soil/solids. This means that it is critical that the method be followed explicitly to achieve an extraction efficiency which approaches that of Soxhlet extraction. Consult Method 3550 for information on the critical aspects of this extraction procedure.

11.2 Suggested chromatographic conditions

Establish the GC operating conditions appropriate for the GC column being utilized and the target analytes specified in the project plan. Optimize the instrumental conditions for resolution of the target analytes and sensitivity. Suggested operating conditions are given below for the columns recommended in Sec. 6.2. The columns listed in this section were the columns used to develop the method performance data and it is not EPA's intent to exclude the use of other columns that may be developed. Laboratories may use these columns or other columns provided that they document method performance data (e.g., chromatographic resolution and sensitivity) that meet the data quality needs of the intended application.

11.2.1 Column 1

Carrier gas (helium) flow rate:	40 mL/min
Temperature program:	
Initial temperature:	45 °C, hold for 3 minutes
Program:	45 °C to 220 °C, at 8 °C/min
Final temperature:	220 °C, hold for 15 minutes.

11.2.2 Column 2

Carrier gas (helium) flow rate:	40 mL/min
Temperature program:	
Initial temperature:	50 °C, hold for 3 minutes
Program:	50 °C to 170 °C, at 6 °C/min
Final temperature:	170 °C, hold for 4 minutes.

11.2.3 Column 3

Carrier gas (helium) flow rate:	15 mL/min
Temperature program:	
Initial temperature:	45 °C, hold for 4 minutes
Program:	45 °C to 220 °C, at 12 °C/min
Final temperature:	220 °C, hold for 3 minutes.

11.2.4 Column 4 (DRO)

Carrier gas (helium) flow rate:	5-7 mL/min
Makeup gas (helium) flow rate:	30 mL/min
Injector temperature:	200 °C
Detector temperature:	340 °C
Temperature program:	
Initial temperature:	45 °C, hold for 3 minutes
Program:	45 °C to 275 °C, at 12 °C/min
Final temperature:	275 °C, hold for 12 minutes.

11.2.5 Column 4 (GRO)

Carrier gas (helium) flow rate:	5-7 mL/min
Makeup gas (helium) flow rate:	30 mL/min
Injector temperature:	200 °C
Detector temperature:	340 °C
Temperature program:	
Initial temperature:	45 °C, hold for 1 minute
1st Ramp:	45 °C to 100 °C at 5 °C/min
2nd Ramp:	100 °C to 275 °C, at 8 °C/min
Final temperature:	275 °C, hold for 5 minutes.

11.2.6 Column 5 (triethylamine only)

Carrier gas (helium) flow rate:	5 mL/min
Makeup gas (helium) flow rate:	30 mL/min
Injector temperature:	200 °C
Detector temperature:	250 °C
Temperature program:	
Initial temperature:	110 °C
Program:	110 °C to 175 °C, at 10 °C/min
Final temperature:	175 °C, hold for 3 minutes.

11.3 Initial calibration

11.3.1 Set up the sample introduction system as outlined in the method of choice (see Sec. 11.1). A separate calibration is necessary for each sample introduction mode because of the differences in conditions and equipment. Establish chromatographic operating parameters that provide instrument performance appropriate for the intended application. Prepare calibration standards using the procedures described above (see Sec. 7.8). The external standard technique is described below. Analysts wishing to use the internal standard technique should refer to Sec. 7.9 and to Method 8000.

11.3.2 External standard calibration procedure for single-component analytes

11.3.2.1 For each analyte and surrogate of interest, prepare calibration standards at a minimum of five different concentrations. For headspace analysis, the standards must be prepared in methanol or organic-free reagent water and then spiked into the organic-free water in the headspace vial. The spiking solutions must be at concentrations which will dilute to the desired standard concentrations when added into the organic-free water in the headspace vials. Otherwise, standards should be made by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with an appropriate solvent. One of the standards should be at a concentration at or below the quantitation limit necessary for the project (based on the concentration in the final volume described in the preparation method, with no dilutions). The concentrations of the other standards should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

11.3.2.2 Introduce each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph. Tabulate peak height or area responses against the mass injected. Calculate the calibration factor (CF) for each single-component analyte as described in Method 8000.

11.3.3 External standard calibration procedure for DRO and GRO

The calibration of DRO and GRO is markedly different from that for single-component analytes. In particular, the response used for calibration must represent the entire area of the chromatogram within the retention time range for the fuel type (DRO or GRO), including the unresolved complex mixture that lies below the individual peaks. See Sec. 11.11 for information on calculating this area.

11.3.3.1 For each fuel type, prepare calibration standards at a minimum of five different concentrations by adding volumes of one or more stock standards to a volumetric flask and diluting to volume with an appropriate solvent (for headspace analysis, follow the instructions in Sec. 11.3.2.1, above). One of the standards should be at a concentration at or below the quantitation limit necessary for the project (based on the concentration in the final volume described in the preparation method, with no dilutions). The concentrations of the other standards should correspond to the expected range of concentrations found in real samples or should define the working range of the detector.

NOTE: Whenever possible, the calibration should be performed using the specific fuel that is contaminating the site (e.g., a sample of the fuel remaining in the tank suspected of leaking). Where such samples are not available or not known, use recently purchased commercially-available fuel. A qualitative screening injection and GC run may be performed to identify unknown fuels.

11.3.3.2 Introduce each calibration standard using the technique that will be used to introduce the actual samples into the gas chromatograph. Determine the area of the response as described in Sec. 11.10. Calculate the calibration factor (CF) for each fuel type as shown below:

$$\text{Calibration Factor} = \frac{\text{Total Area within Retention Time Range}}{\text{Mass injected (nanograms)}}$$

11.3.4 Calibration linearity

The linearity of the calibration must be assessed. This applies to both the single-component analytes and the fuel types.

11.3.4.1 If the percent relative standard deviation (%RSD) of the calibration factors is less than 20% over the working range, then linearity through the origin can be assumed, and the average calibration factor can be used in place of a calibration curve.

11.3.4.2 If the % RSD is more than 20% over the working range, linearity through the origin cannot be assumed. See Method 8000 for other calibration options that may be employed, which may include: a linear calibration not through the origin or a non-linear calibration model (e.g., a polynomial equation).

11.4 Retention time windows

Single-component target analytes (see Sec. 1.1) are identified on the basis of retention time windows. GRO and DRO are distinguished on the basis of the ranges of retention times for characteristic components in each type of fuel.

11.4.1 Before establishing retention time windows, make sure that the chromatographic system is functioning reliably and that the operating parameters have been optimized for the target analytes and surrogates in the sample matrix to be analyzed. Establish the retention time windows for single component target analytes using the procedure described in Sec. 11.0 of Method 8000.

11.4.2 The retention time range for GRO is defined during initial calibration. Two specific gasoline components are used to establish the range, 2-methylpentane and 1,2,4-trimethylbenzene. Use the procedure described in Sec. 11.0 of Method 8000 to establish the retention time windows for these two components. The retention time range is then calculated based on the lower limit of the RT window for the first eluting component and the upper limit of the RT window for the last eluting component.

11.4.3 The retention time range for DRO is defined during initial calibration. The range is established from the retention times of the C_{10} and C_{28} alkanes. Use the procedure described in Sec. 11.0 of Method 8000 to establish the retention time windows for these two components. The retention time range is then calculated based on the lower limit of the RT window for the first eluting component and the upper limit of the RT window for the last eluting component.

11.4.4 If this method is expanded to address other fuel types or petroleum fractions, then the analyst must establish appropriate retention time ranges for the boiling point range or carbon number range used to define each additional fuel type or petroleum fraction. Use the procedure described in Sec. 11.0 of Method 8000 to establish the retention time windows.

11.5 Calibration verification

11.5.1 The initial calibration and retention times must be verified at the beginning of each 12-hour work shift, at a minimum. When individual target analytes are being analyzed, verification is accomplished by the analysis of one or more calibration standards (normally mid-concentration, but a concentration at or near the action level may be more appropriate) that contain all of the target analytes and surrogates. When petroleum hydrocarbons are being analyzed, verification is accomplished by the measurement of the fuel standard and the hydrocarbon retention time standard. Additional analyses of the verification standard(s) throughout a 12-hour shift are strongly recommended, especially for samples that contain visible concentrations of oily material. See Sec. 11.0 of Method 8000 for more detailed information on calibration verification.

11.5.2 Calculate the % difference as detailed in Sec. 11.0 of Method 8000. If the response for any analyte is within ±20% of the response obtained during the initial calibration, then the initial calibration is considered still valid, and the analyst may continue to use the mean CF or RF values from the initial calibration to quantitate sample results. If the response for any analyte varies from the predicted response by more than ±20%, corrective action must be taken to restore the system or a new calibration curve must be prepared for that compound.

11.5.3 All target analytes, surrogates, and/or *n*-alkanes in the calibration verification analyses must fall within previously established retention time windows. If the retention time of any analyte does not fall within the established window, then corrective action must be taken to restore the system or a new calibration curve must be prepared for that compound.

11.5.4 Solvent blanks and any method blanks should be run with calibration verification analyses to confirm that laboratory contamination does not cause false positive results.

11.6 Gas chromatographic analysis

11.6.1 Samples are analyzed in a set referred to as an analytical sequence. The sequence begins with calibration verification followed by sample extract analyses. Additional analyses of the verification standard(s) throughout a 12-hour shift are strongly recommended, especially for samples that contain visible concentrations of oily material. A verification standard is also necessary at the end of a set (unless internal standard calibration is used). The sequence ends when the set of samples has been injected or when retention time and/or % difference QC criteria are exceeded.

If the criteria are exceeded, inspect the gas chromatographic system to determine the cause and perform whatever maintenance is necessary before recalibrating and proceeding with sample analysis. All sample analyses performed using external standard calibration must be bracketed with acceptable data quality analyses (e.g., calibration and retention time criteria). Therefore, all samples that fall between the standard that failed to meet the acceptance criteria and the preceding standard that met the acceptance criteria must be reanalyzed. Samples analyzed using internal standard calibration need not be bracketed (see Method 8000).

11.6.2 Samples are analyzed with the same instrument configuration as is used during calibration. When using Method 5030 for sample introduction, analysts are cautioned that opening a sample vial or drawing an aliquot from a sealed vial (thus creating headspace) will compromise samples analyzed for volatiles. Therefore, it is recommended that analysts prepare two aliquots for purge-and-trap analysis. The second aliquot can be stored for 24 hours to ensure that an uncompromised sample is available for analysis or dilution, if the analysis of the first aliquot is unsuccessful or if results exceed the calibration range of the instrument. Distillates from Method 5031 may be split into two aliquots and held at 4 °C prior to analysis. It is recommended that the distillate be analyzed within 24 hours of distillation. Distillates must be analyzed within 7 days of distillation.

11.6.3 Sample concentrations are calculated by comparing the sample response with the response from the initial calibration of the system (see Sec. 11.3). Therefore, if the sample response exceeds the limits of the initial calibration range, a dilution of the sample or sample extract must be analyzed. For volatile organic analyses of aqueous samples, the dilution must be performed on a second aliquot of the sample which has been properly sealed and stored prior to use and reanalysis. Samples and/or sample extracts should be diluted so that all peaks are on scale, as overlapping peaks are not always evident when peaks are off scale. Computer reproduction of chromatograms, manipulated to ensure all peaks are on scale over a 100-fold range, is acceptable as long as calibration limits are not exceeded. Peak height measurements are recommended over peak area integration when overlapping peaks cause errors in area integration.

11.6.4 Tentative identification of a single-component analyte occurs when a peak from a sample extract falls within the daily retention time window. Confirmation may be necessary on a second column or by GC/MS. Since the flame ionization detector is non-specific, it is highly recommended that GC/MS confirmation be performed on single-component analytes unless historical data are available to support the identification(s). See Method 8000 for additional information on confirmation.

11.6.5 Second-column confirmation is generally not necessary for petroleum hydrocarbon analysis. However, if analytical interferences are indicated, analysis using the second GC column may be necessary. Also, the analyst must ensure that the sample hydrocarbons fall within the retention time range established during the initial calibration.

NOTE: The identification of fuels, especially gasoline, is complicated by their inherent volatility. The early eluting compounds in fuels are obviously the most volatile and the most likely to have weathered unless the samples were taken immediately following a spill. The most highly volatile fraction of gasoline constitutes 50% of the total peak area of a gasoline chromatogram. This fraction is the least likely to be present in an environmental sample or may be present at only very low concentration in relation to the remainder of a gasoline chromatogram.

11.6.6 The performance of the entire analytical system should be checked every 12 hours, using data gathered from analyses of blanks, standards, and samples. Significant peak tailing must be corrected. Tailing problems are generally traceable to active sites on the column, cold spots in a GC, the detector operation, or leaks in the system. See Sec. 11.8 for GC/FID system maintenance. Follow manufacturer's instructions for maintenance of the introduction device.

11.7 Screening

11.7.1 This method can be used with a single-point calibration for screening samples prior to GC/MS analyses (e.g., Methods 8260 and 8270). Such screening can reduce GC/MS down-time when highly-contaminated samples are analyzed.

11.7.2 When this method is used for screening, it is recommended that the same sample introduction device (e.g., purge-and-trap versus direct injection) that is used for the subsequent GC/MS analyses also be used for the screening analysis. This will improve the correlation between the results and make the screening results more useful in predicting those samples that may overload the GC/MS system. However, other sample introduction techniques may be employed as well.

11.7.3 Establish that the system response and chromatographic retention times are stable. Analyze the high-point GC/MS calibration standard.

11.7.4 Analyze samples or sample extracts. Compare peak heights in the sample chromatograms with the high-point standard to establish that no compound with the same retention time as a target analyte exceeds the calibration range of the GC/MS system.

NOTE: The FID is much less sensitive to halogenated compounds than the MS detector. As a result, a simple peak height comparison for such compounds in the GC/MS standard may underestimate the actual concentration of halogenated compounds. When using this method as a screening tool, such an underestimate could lead to GC/MS results over the calibration range or result in contamination of the GC/MS system. Therefore, the analyst should exercise caution when screening samples that also contain halogenated compounds.

11.7.5 There are no formal QC requirements applied to screening analyses using this method. However, it is recommended that the high-point standard be run at least once every 12 hours to confirm the stability of the instrument response and chromatographic retention times. The analyst should consider the costs associated with making the wrong decision from the screening results (e.g., GC/MS instrument down-time and maintenance) and use appropriate judgment.

11.8 Instrument Maintenance

11.8.1 Injection of sample extracts from waste sites often leaves a high boiling residue in the injection port area, splitters (when used), and the injection port end of the chromatographic column. This residue affects chromatography in many ways (i.e., peak tailing, retention time shifts, analyte degradation, etc.) and, therefore, instrument maintenance is very important. Residue buildup in a splitter may limit flow through one leg and therefore change the split ratios. If this occurs during an analytical run, the quantitative data may be incorrect. Proper cleanup techniques will minimize the problem and instrument QC will indicate when instrument maintenance is necessary.

11.8.2 Recommended chromatograph maintenance

Corrective measures may require any one or more of the following remedial actions. Also see Sec. 11.0 in Method 8000 for additional guidance on corrective action for capillary columns and the injection port.

11.8.2.1 Splitter connections - For dual columns which are connected using a press-fit Y-shaped glass splitter or a Y-shaped fused-silica connector, clean and deactivate the splitter or replace with a cleaned and deactivated splitter. Break off the first few inches (up to one foot) of the injection port side of the column. Remove the columns and solvent backflush according to the manufacturer's instructions. If these procedures fail to eliminate the degradation problem, it may be necessary to deactivate the metal injector body and/or replace the columns.

11.8.2.2 Column rinsing - The column should be rinsed with several column volumes of an appropriate solvent. Both polar and nonpolar solvents are recommended. Depending on the nature of the sample residues expected, the first rinse might be water, followed by methanol and acetone; methylene chloride is a satisfactory final rinse and in some cases may be the only solvent necessary. The column should then be filled with methylene chloride and allowed to remain flooded overnight to allow materials within the stationary phase to migrate into the solvent. The column is then flushed with fresh methylene chloride, drained, and dried at room temperature with a stream of ultrapure nitrogen passing through the column.

11.9 Calculations and data handling

Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

11.10 The concentration of each analyte in the sample may be determined by calculating the amount of standard purged or injected, from the peak response, using the mean CF or RF from the initial calibration, or another appropriate calibration model (see Method 8000).

11.11 While both diesel fuel and gasoline contain a large number of compounds that will produce well-resolved peaks in a GC/FID chromatogram, both fuels contain many other components that are not chromatographically resolved. This unresolved complex mixture results in the "hump" in the chromatogram that is characteristic of these fuels. In addition, although the resolved peaks are important for the identification of the specific fuel type, the area of the unresolved complex mixture contributes a significant portion of the area of the total response.

11.11.1 For the analysis of DRO, sum the area of all peaks eluting between C_{10} and C_{28}. This area is generated by projecting a horizontal baseline between the retention times of C_{10} and C_{28}.

11.11.2 Because the chromatographic conditions employed for DRO analysis can result in significant column bleed and a resulting rise in the baseline, it is appropriate to perform a subtraction of the column bleed from the area of the DRO chromatogram. In order to accomplish this subtraction, a methylene chloride blank should be analyzed during each 12-hour analytical shift during which samples are analyzed for DRO. The area of this chromatogram is measured in the same fashion as is used for samples (see Sec. 12.3.1), by projecting a horizontal baseline across the retention time range for DRO. This area is then subtracted from the area measured for the sample and the difference in areas is used to calculate the DRO concentration, using the equations in Method 8000.

11.11.3 For the analysis of GRO, sum the areas of all peaks eluting between 2-methylpentane and 1,2,4-trimethylbenzene. This area is used to calculate the GRO concentration, using the equations in Method 8000. Column bleed subtraction is not generally necessary for GRO analysis.

11.12 Refer to Method 8000, Sec. 11.0, for the calculation formulae. The formulae cover external and internal standard calibration, aqueous and non-aqueous samples, and linear and non-linear calibrations.

12.0 DATA ANALYSIS AND CALCULATIONS

See Sec. 11.0 for information on data analysis and calibration.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

13.2 Example method performance data for non-purgeable volatiles prepared using the azeotropic microdistillation technique from Method 5031 are included in Tables 1, 3 and 4 for aqueous matrices and in Tables 2 and 5 for solid matrices. Typical chromatograms are included in Figs. 4 and 5. These data are for illustrative purposes only.

13.3 Example method performance information are provided in Tables 6 and 7 for diesel fuel spiked into soil as are chromatograms of GRO and DRO standards in Figures 1 to 3.. These data are for illustrative purposes only.

13.4 Table 8 contains example precision and bias data for the analysis of triethylamine. Reagent water was spiked with triethylamine at 1.0 μg/L and analyzed by direct aqueous injection in a GC/FID equipped with an HP Basic Wax column (30-m x 0.53-mm ID). These data are for illustrative purposes only.

13.5 Table 9 contains example single-laboratory data on the pressurized fluid extraction of diesel range organics (DRO) from three types of soil (sand, loam, and clay). The soils were spiked at two levels of DRO, approximately 5 mg/kg each and approximately 2000 mg/kg. Seven replicates of each level and soil type were extracted using pressurized fluid extraction (Method 3545), using a mixture of methylene chloride and acetone (1:1). The data are taken from Reference 8 and are for illustrative purposes only. This extraction technique may be applicable to other analyte classes, fuel types, or petroleum fractions (see Sec. 1.2.3).

13.6 This method was the determinative technique used by one of the three laboratories participating in the study of headspace analysis of oxygenated gasoline contaminated groundwater samples. Please refer to Secs. 13.0 and 17.0 of Method 5021 for discussion and the results of that study.

13.7 This method was used in combination with Method 5021 to analyze a standard with several gasoline components, including MTBE and 2-methylpentane. As can be seen from the chromatogram in Figure 6, the two analytes can be resolved quite well (column: Restek 502.2 105m x 0.53 um, 3 df).

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th St., N.W. Washington, D.C. 20036, (202) 872-4477.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

16.0 REFERENCES

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10. USEPA OUST, *Environmental Fact Sheet: Analytical Methods for Fuel Oxygenates,* EPA 510-F-03-001, April, 2003, http://www.epa.gov/OUST/mtbe/omethods.pdf.

11. White, H., Lesnik, B., and Wilson, J. T., “Analytical Methods for Fuel Oxygenates”, *LUSTLine* (Bulletin #42), October, 2002, http://www.epa.gov/oust/mtbe/LL42Analytical.pdf

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

TABLE 1

EXAMPLE LOWER LIMITS OF DETECTION FOR NON-PURGEABLE VOLATILE COMPOUNDS IN AQUEOUS MATRICES BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

	Lower Quantitation Limit (µg/L)[a]		
Analyte	**Reagent Water**	**Ground Water**	**TCLP Leachate**
Acetone[b]	48	16	63
Acetonitrile	15	6	14
Acrolein	13	15	7
Acrylonitrile	8	9	14
1-Butanol	14	8	7
t-Butyl alcohol	8	7	17
1,4-Dioxane	12	15	16
Ethanol	18	12	13
Ethyl acetate	9	8	16
Ethylene oxide	8	9	10
Isobutyl alcohol	11	8	4
Isopropyl alcohol	18	17	7
Methanol	21	21	22
Methyl ethyl ketone	4	5	9
Methyl isobutyl ketone	4	2	8
2-Pentanone	2	2	7
1-Propanol	--	7	--
Propionitrile	10	6	13
Pyridine	11	9	21

a Derived from analysis of seven aliquots of water spiked at 25 µg/L, using internal standard calibration. **Lower quantitation limits provided in SW-846 are for illustrative purposes and may not always be achievable.** Laboratories should establish their own in-house lower quantitation limits, if necessary to document method performance

b Problematic due to transient laboratory contamination.

TABLE 2

EXAMPLE LOWER LIMITS OF DETECTION FOR NON-PURGEABLE VOLATILE COMPOUNDS IN SOLID MATRICES BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

Analyte	**Lower Quantitation Limit (mg/kg)**	
	Incinerator Ash	**Kaolin**
Acrylonitrile	0.42	0.09
1-Butanol	0.23	0.09
t-Butyl alcohol	0.34	0.13
1,4-Dioxane	0.31	0.16
Ethanol	0.47	0.19
Ethyl acetate	0.18	0.07
Isopropyl alcohol	0.40	0.19
Methanol	0.46	0.31
Methyl ethyl ketone	0.27	0.12
Methyl isobutyl ketone	0.12	0.05
2-Pentanone	0.16	0.07
Pyridine	0.20	0.08

Derived from analysis of seven aliquots of incinerator ash and kaolin spiked at 0.50 mg/kg, using internal standard calibration. **Lower quantitation limits provided in SW-846 are for illustrative purposes and may not always be achievable.** Laboratories should establish their own in-house lower quantitation limits, if necessary to document method performance

TABLE 3

EXAMPLE METHOD PERFORMANCE DATA FOR NON-PURGEABLE VOLATILES IN GROUND WATER BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

Analyte	Low Concentration[a]		Med. Concentration[b]		High Concentration[c]	
	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD
Acetone[e]	126	17	N/A	--	N/A	--
Acetonitrile	147	5	105	8	92	9
Acrolein	146	13	120	27	80	20
Acrylonitrile	179	7	143	28	94	21
1-Butanol	127	8	86	8	90	9
t-Butyl alcohol	122	7	N/A	--	N/A	--
1,4-Dioxane	124	16	96	10	99	8
Ethanol	152	10	N/A	--	N/A	--
Ethyl Acetate	142	7	135	33	92	25
Ethylene oxide	114	10	N/A	--	N/A	--
Isobutyl alcohol	122	8	87	13	89	13
Isopropyl alcohol	167	13	N/A	--	N/A	--
Methanol	166	14	94	9	95	7
Methyl ethyl ketone	105	6	N/A	--	N/A	--
Methyl isobutyl ketone	66	4	N/A	--	N/A	--
2-Pentanone	94	3	N/A	--	N/A	--
1-Propanol	N/A	--	91	7	91	7
Propionitrile	135	5	102	14	90	14
Pyridine	92	12	N/A	--	N/A	--

[a] 25 µg/L spikes, using internal standard calibration.
[b] 100 µg/L spikes, using internal standard calibration.
[c] 750 µg/L spikes, using internal standard calibration.
[d] Mean of 7 replicates.
[e] Problematic, due to transient laboratory contamination.

N/A = Data not available

TABLE 4

EXAMPLE METHOD PERFORMANCE DATA FOR NON-PURGEABLE VOLATILES IN TCLP LEACHATE BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

Analyte	Low Concentration[a]		Med. Concentration[b]		High Concentration[c]	
	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD	Mean Rec[d]	%RSD
Acetone[e]	99	91	N/A	--	N/A	--
Acetonitrile	107	17	111	10	95	11
Acrolein	88	10	109	29	87	41
Acrylonitrile	133	13	123	29	103	38
1-Butanol	119	7	89	12	86	8
t-Butyl alcohol	70	31	N/A	--	N/A	--
1,4-Dioxane	103	20	103	16	102	7
Ethanol	122	13	N/A	--	N/A	--
Ethyl Acetate	164	12	119	29	107	41
Ethylene oxide	111	12	N/A	--	N/A	--
Isobutyl alcohol	115	4	86	13	82	13
Isopropyl alcohol	114	8	N/A	--	N/A	--
Methanol	107	10	102	6	N/A	--
Methyl ethyl ketone	87	13	N/A	--	N/A	--
Methyl isobutyl ketone	78	13	N/A	--	N/A	--
2-Pentanone	101	8	N/A	--	N/A	--
1-Propanol	N/A	--	98	10	89	7
Propionitrile	100	16	100	11	90	17
Pyridine	46	59	N/A	--	N/A	--

[a] 25 µg/L spikes, using internal standard calibration.
[b] 100 µg/L spikes, using internal standard calibration.
[c] 750 µg/L spikes, using internal standard calibration.
[d] Mean of 7 replicates.
[e] Problematic, due to transient laboratory contamination.

N/A = Data not available

TABLE 5

EXAMPLE METHOD PERFORMANCE DATA FOR NON-PURGEABLE VOLATILE COMPOUNDS IN SOLID MATRICES BY AZEOTROPIC MICRODISTILLATION (METHOD 5031)

	Incinerator Ash				**Kaolin**			
	Low Conc.[a]		**High Conc.[b]**		**Low Conc.[a]**		**High Conc.[b]**	
	Mean Rec[c]	**%RSD**	**Mean Rec[c]**	**%RSD**	**Mean Rec[c]**	**%RSD**	**Mean Rec[c]**	**%RSD**
Acrylonitrile	50	53	10	31	102	6	12	52
1-Butanol	105	14	61	12	108	5	58	25
t-Butyl alcohol	101	21	60	13	97	9	59	23
1,4-Dioxane	106	19	48	18	105	10	48	25
Ethanol	117	25	52	20	108	11	48	24
Ethyl acetate	62	19	39	12	90	5	41	25
Isopropyl alcohol	119	21	61	15	108	11	58	24
Methanol	55	53	33	28	117	17	37	22
Methyl ethyl ketone	81	21	40	12	91	8	42	20
Methyl isobutyl ketone	68	11	57	14	71	5	55	23
2-Pentanone	79	13	54	10	91	5	54	19
Pyridine	52	24	44	20	50	10	49	31

[a] 0.5 mg/kg spikes, using internal standard calibration.
[b] 25 mg/kg spikes, using internal standard calibration.
[c] Mean of seven replicates.

TABLE 6

EXAMPLE RESULTS FROM ANALYSIS OF LOW AROMATIC DIESEL BY GC/FID
(5 replicates per test)

Spike Concentration	Analysis Results
12.5 ppm	ND
75 ppm	54 ± 7 ppm
105 ppm	90 ± 15 ppm
150 ppm	125 ± 12 ppm
1000 ppm	960 ± 105 ppm

ND = Not detected

Samples were prepared using 2-g aliquots of sandy loam soil spiked with known amounts of low aromatic diesel. Low aromatic diesel is sold in California. It was purchased for this study at a gas station in San Diego, California. Extractions were accomplished using methylene chloride as a solvent (Method 3550, high concentration option).

TABLE 7

EXAMPLE RESULTS FROM ANALYSIS OF DIESEL BY GC/FID
(5 replicates per test)

Spike Concentration	Analysis Results
25 ppm	51 ± 6 ppm
75 ppm	76 ± 8 ppm
125 ppm	99 ± 5 ppm
150 ppm	160 ± 10 ppm

Samples were prepared using 10-g aliquots of sandy loam soil spiked with known amounts of regular #2 diesel purchased at a gas station in Northern Virginia. Extractions were accomplished using methylene chloride as a solvent (Method 3550).

TABLE 8

EXAMPLE TRIETHYLAMINE PERFORMANCE DATA FOR SPIKED REAGENT WATER
(Analyses by Direct Aqueous Injection)

Triethylamine Concentration (μg/L)								
Spike Conc.	**Rep. 1**	**Rep. 2**	**Rep. 3**	**Rep. 4**	**Rep. 5**	**Rep. 6**	**Rep. 7**	**Mean**
1.00	1.12	1.17	1.14	1.20	1.19	1.18	1.18	1.169
Mean Recovery	117%	S.D.	0.0288 μg/L					

The estimated quantitation limit was derived from the analyses of seven aliquots of water spiked at 1.00 μg/L, using external standard calibration, on a 30-m, 0.53-mm ID, HP Basic Wax GC column. A 1-μL injection volume was used. **Lower quantitation limits provided in SW-846 are for illustrative purposes and may not always be achievable.** Laboratories should establish their own in-house lower quantitation limits, if necessary to document method performance.

Data are taken from Reference 7.

TABLE 9

SINGLE-LABORATORY DATA FOR PRESSURIZED FLUID EXTRACTION (METHOD 3545)
OF DIESEL RANGE ORGANICS FROM THREE SOIL MATRICES

	DRO Concentration in mg/kg									
Matrix and Spiking Level	**Rep. 1**	**Rep. 2**	**Rep. 3**	**Rep. 4**	**Rep. 5**	**Rep. 6**	**Rep. 7**	**Mean**	**Mean Recovery (%)**	**%RSD**
Low Sand	3.2	8.2	5.9	6.3	7.0	7.7	6.4	6.4	127	25.4
Low Loam	6.5	6.0	7.9	5.1	6.9	9.5	6.4	6.9	138	20.7
Low Clay	4.3	5.8	5.7	8.6	5.4	7.5	7.6	6.4	128	23.6
High Sand	1850	1970	2030	2390	2210	2400	2430	2183	108	10.8
High Loam	1790	1870	1860	1970	1790	1990	1990	1894	94	4.7
High Clay	1910	1890	1990	2860	2880	2150	2040	2246	112	19.4

Low level samples were spiked with approximately 5 mg/kg of DRO.

High level samples were spiked with approximately 2000 mg/kg of DRO.

Seven replicates of each sample were extracted and analyzed by GC/FID.

Data are taken from Reference 8.

FIGURE 1

EXAMPLE CHROMATOGRAM OF A 300 PPM GASOLINE STANDARD

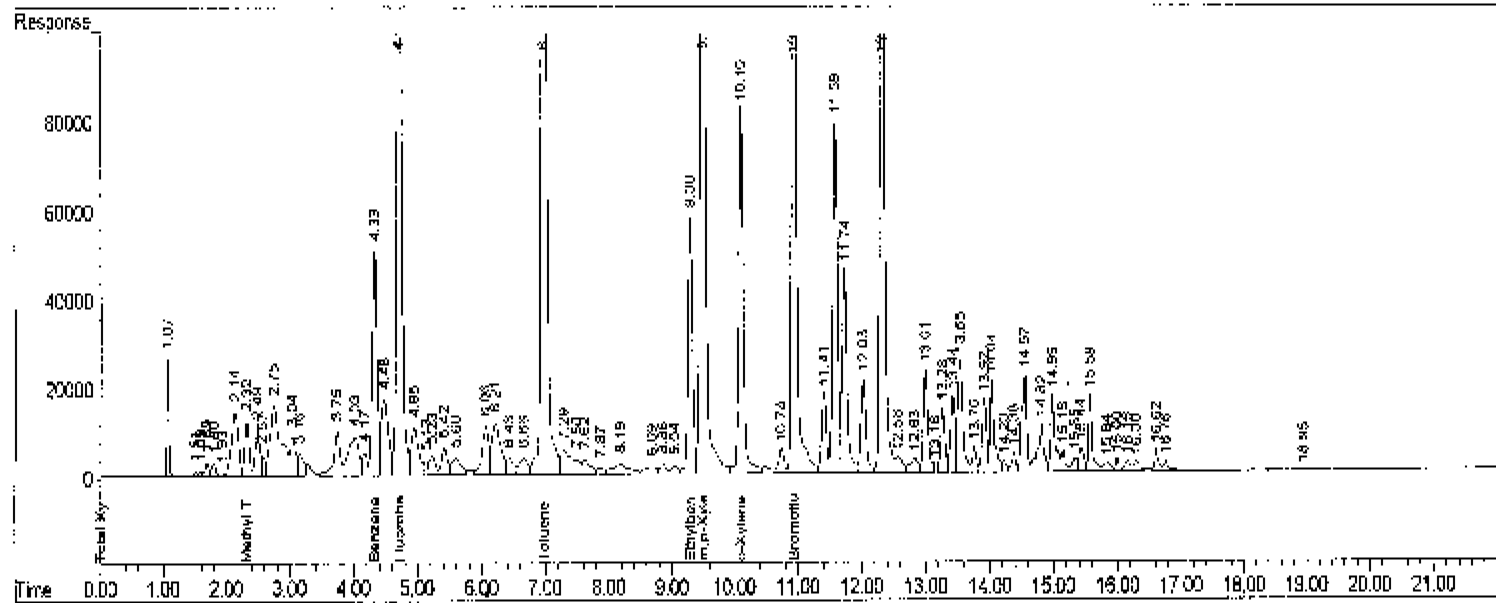


FIGURE 2

EXAMPLE CHROMATOGRAM OF A 30 PPM DIESEL STANDARD

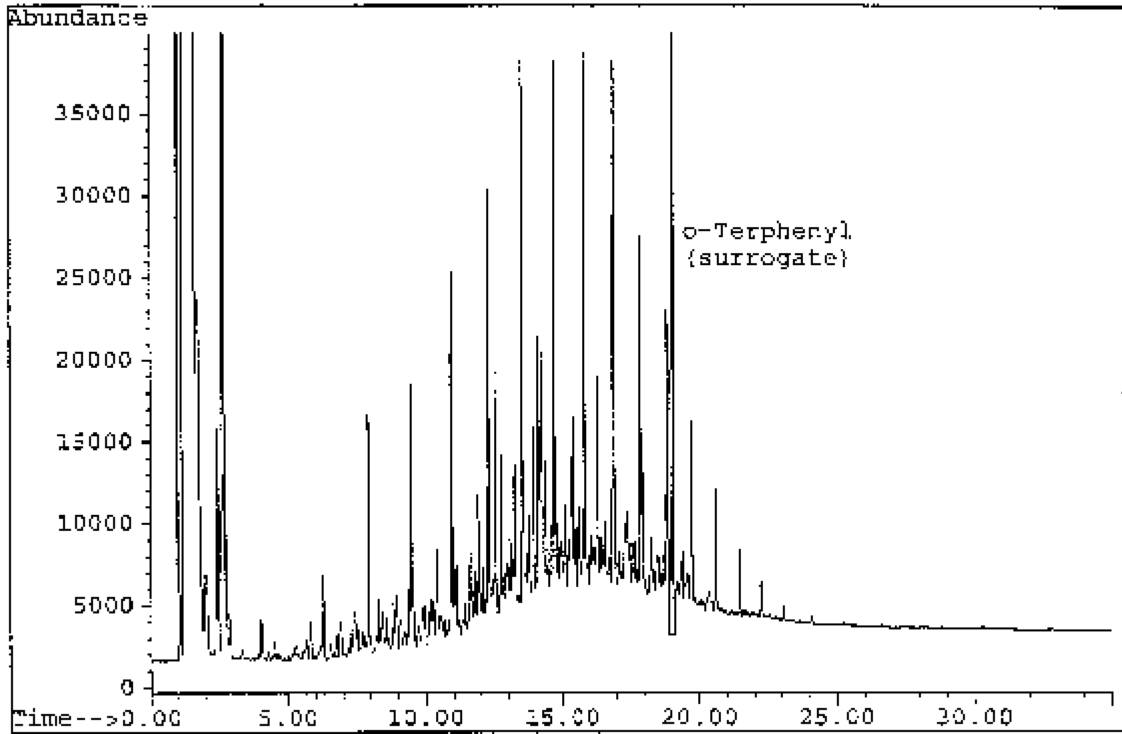


FIGURE 3

EXAMPLE CHROMATOGRAM OF A 30 PPM DIESEL STANDARD WITH THE BASELINE PROJECTED BETWEEN C_{10} AND C_{28}

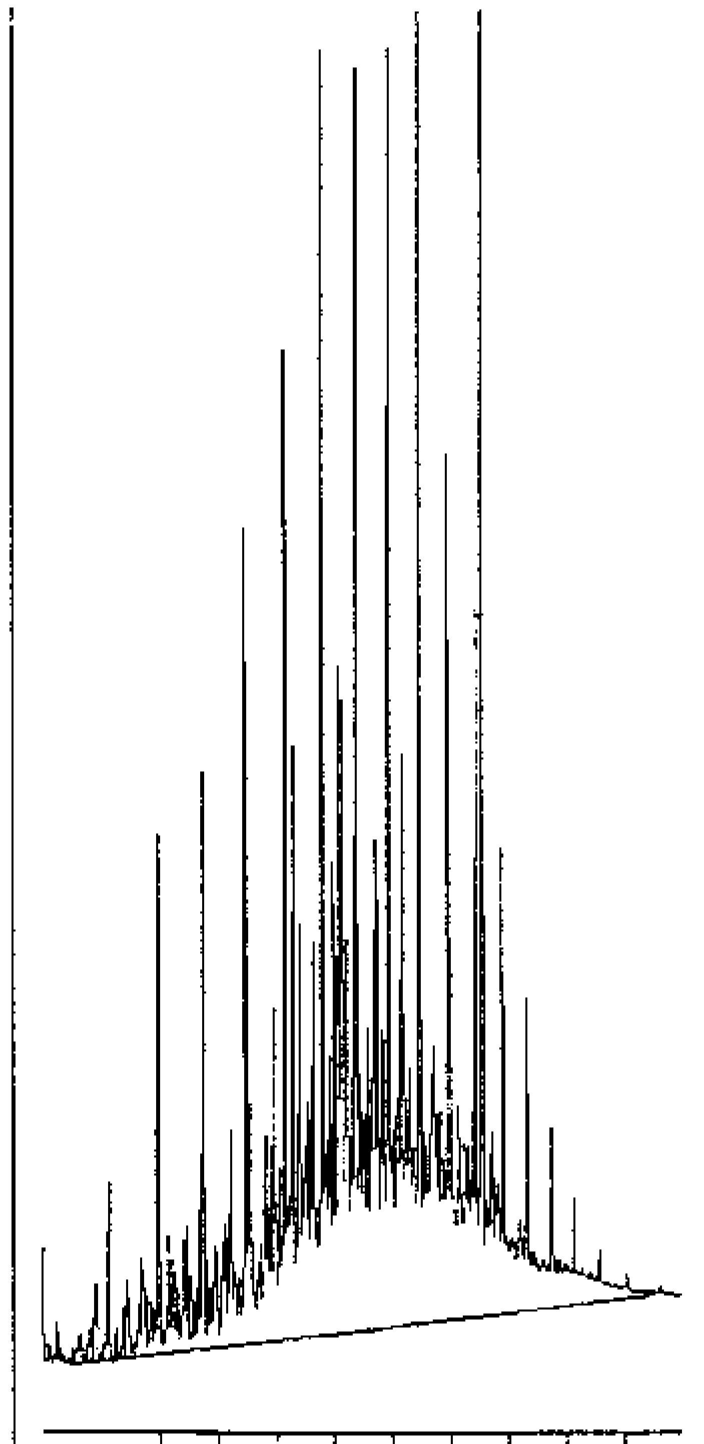
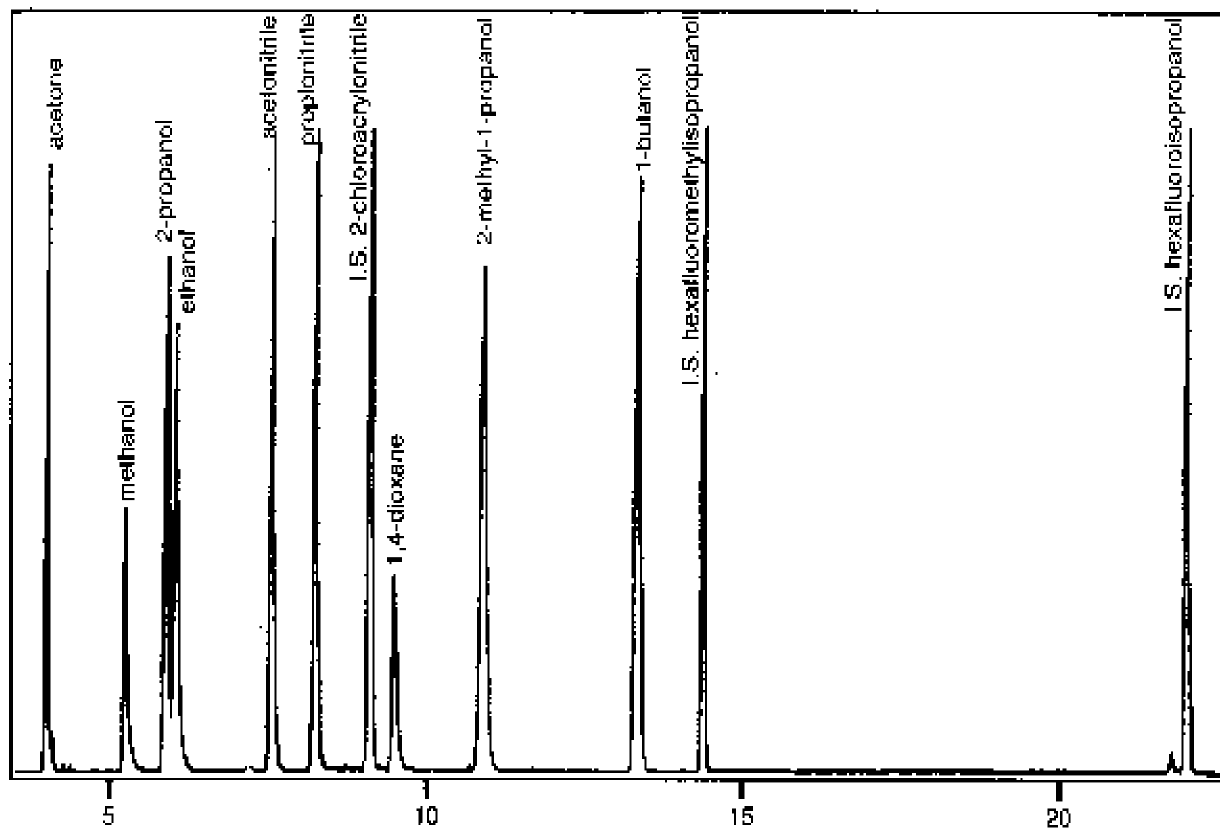


FIGURE 4

EXAMPLE CHROMATOGRAM OF SEVERAL NONPURGEABLE VOLATILE COMPOUNDS IN SPIKED REAGENT WATER USING AZEOTROPIC MICRODISTILLATION (METHOD 5031)

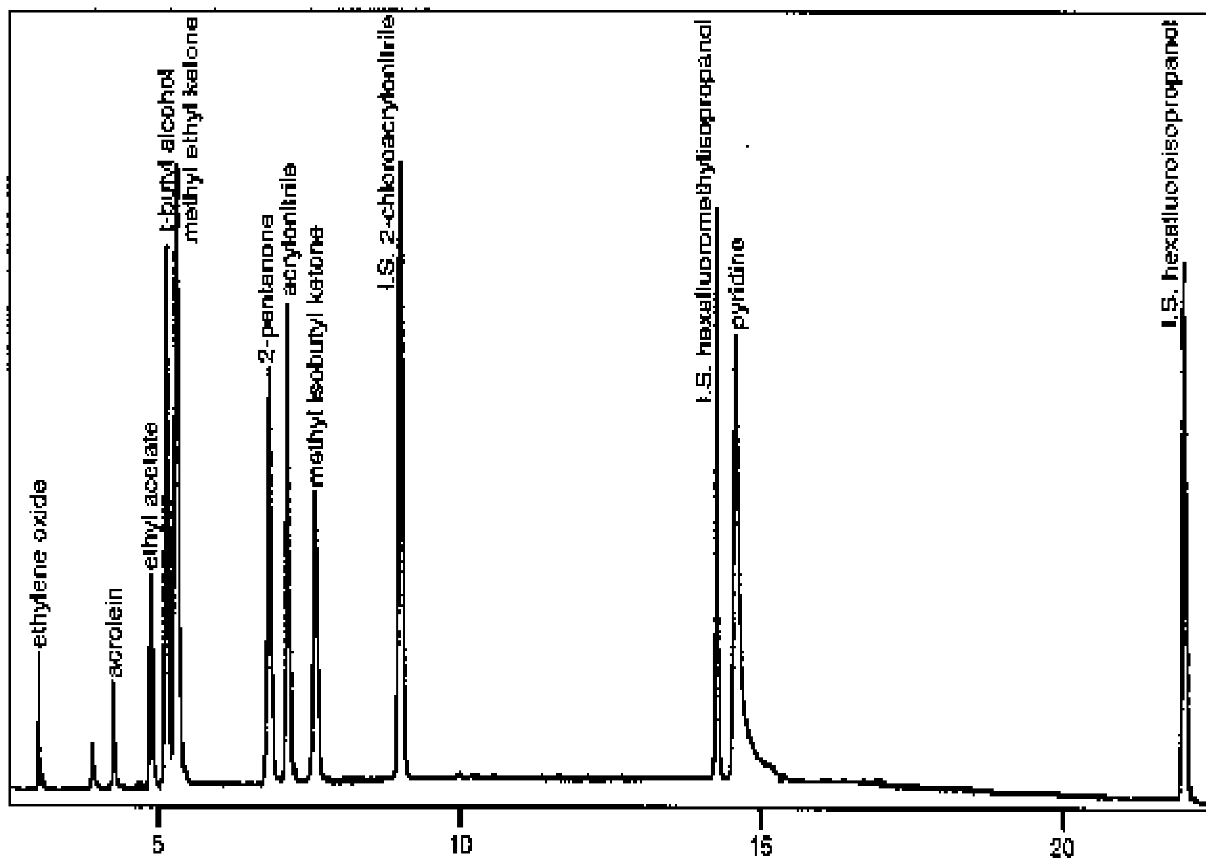


Mix 1: Analytes distilled at 0.25 mg/L, internal standards at 2.5 mg/L

GC Column: J&W DB-Wax column with 0.53-mm ID

Temperature program: 30 °C for 2 min.
3 °C/min. to 100 °C and held for 0 min.
25 °C/min. to 200 °C and held for 4 min.

EXAMPLE CHROMATOGRAM OF SEVERAL NONPURGEABLE VOLATILE COMPOUNDS IN SPIKED REAGENT WATER USING AZEOTROPIC MICRODISTILLATION (METHOD 5031)



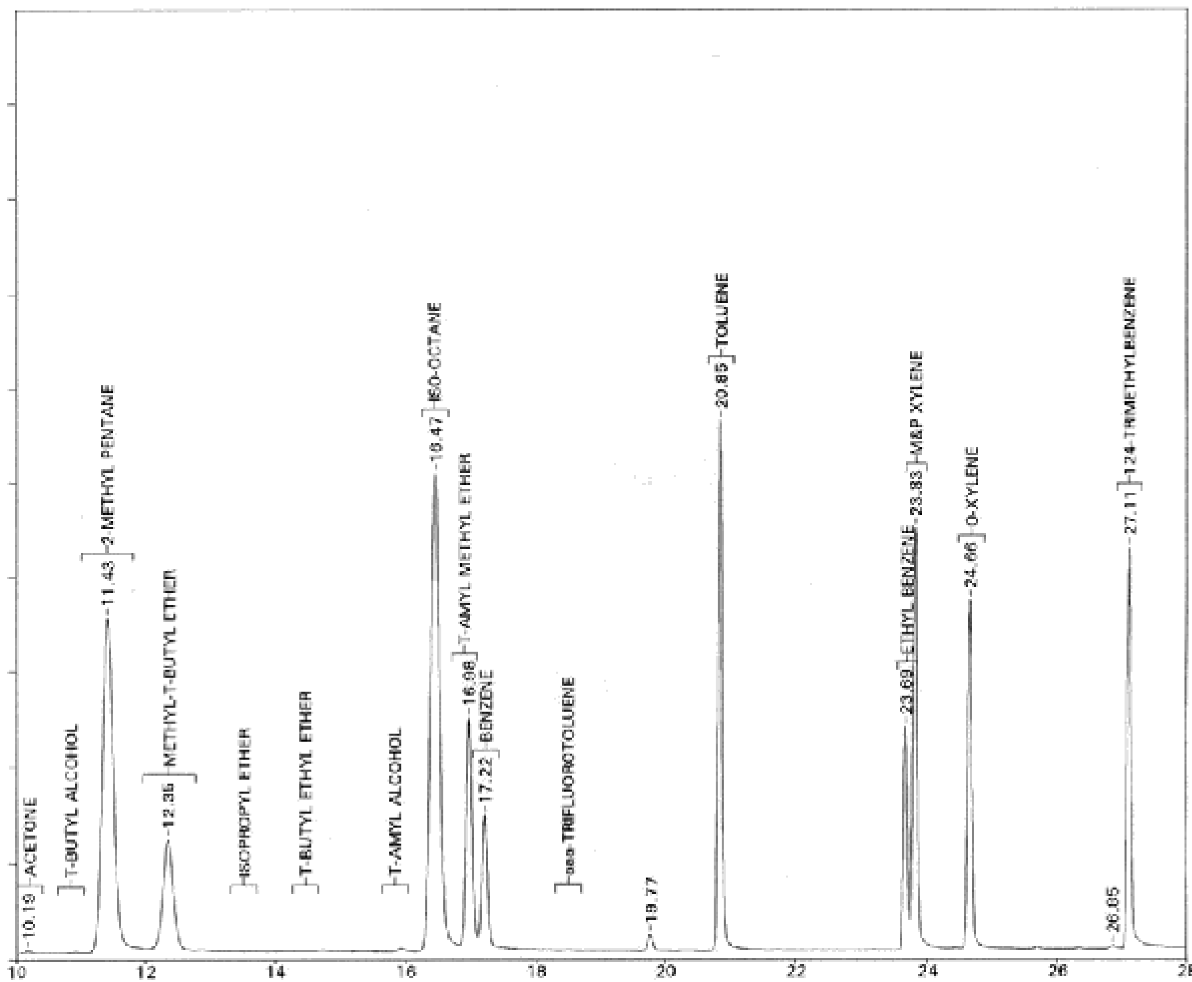
Mix 2: Analytes distilled at 0.25 mg/L, internal standards at 2.5 mg/L

GC Column: J&W DB-Wax column with 0.53-mm ID

Temperature program:
30 °C for 2 min.
3 °C/min. to 100 °C and held for 0 min.
25 °C/min. to 200 °C and held for 4 min.

FIGURE 6

EXAMPLE CHROMATOGRAM OF MULTI-COMPONENT MIXTURE



METHOD 8260D
VOLATILE ORGANIC COMPOUNDS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

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Disclaimer

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required methods used for the analysis of method-defined parameters, are intended to be guidance methods that contain general information on how to perform an analytical procedure or technique. A laboratory can use this guidance as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data referenced in this method are for guidance purposes only, and are not intended to be and must not be used as absolute quality control (QC) acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

1.1 This method is used to determine volatile organic compounds (VOCs) in a variety of solid waste matrices. This method is applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, ground and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments. The following analytes have been determined by this method:

Analytes and Appropriate Preparation Techniques

Compound	CAS No.[a]	5030	5035	5031	5032	5021	5041	Direct Inject
Acetone	67-64-1	*	*	✓	✓	-	✓	✓
Acetonitrile	75-05-8	*	*	✓	-	-	-	✓
Acrolein (Propenal)	107-02-8	*	*	✓	-	-	-	✓
Acrylonitrile	107-13-1	*	*	✓	*	-	✓	✓
Allyl alcohol	107-18-6	*	-	✓	-	-	-	✓
Allyl chloride	107-05-1	✓	*	-	-	-	-	✓
t-Amyl ethyl ether (TAEE, 4,4-Dimethyl-3-oxahexane)	919-94-8	*	*	-	-	✓*	-	✓
t-Amyl methyl ether (TAME)	994-05-8	*	*	-	-	✓*	-	✓
Benzene	71-43-2	✓	✓	-	✓	✓	✓	✓
Benzyl chloride	100-44-7	*	✓	-	-	-	-	✓
Bromoacetone	598-31-2	*	-	-	-	-	-	✓
Bromobenzene	108-86-1	✓	✓	-	✓	-	-	-
Bromochloromethane	74-97-5	✓	✓	-	✓	✓	✓	✓
Bromodichloromethane	75-27-4	✓	✓	-	✓	✓	✓	✓
Bromoform	75-25-2	*	*	-	✓	✓	✓	✓
Bromomethane	74-83-9	*	*	-	✓	✓	✓	✓
n-Butanol (1-Butanol, *n*-Butyl alcohol)	71-36-3	*	*	✓	-	✓	-	✓
2-Butanone (MEK)	78-93-3	*	*	✓	✓	-	-	✓
t-Butyl alcohol	75-65-0	*	*	✓	-	✓*	-	✓
n-Butylbenzene	104-51-8	✓	✓	-	✓	-	-	-
sec-Butylbenzene	135-98-8	✓	✓	-	✓	-	-	-
tert-Butylbenzene	98-06-6	✓	✓	-	✓	-	-	-
Carbon disulfide	75-15-0	*	*	-	✓	✓	✓	✓
Carbon tetrachloride	56-23-5	✓	✓	-	✓	✓	✓	✓
Chloral hydrate	302-17-0	*	-	-	-	-	-	✓
Chlorobenzene	108-90-7	✓	✓	-	✓	✓	✓	✓
1-Chlorobutane	109-69-3	✓	✓	-	✓	-	-	-
Chlorodibromomethane (Dibromochloromethane)	124-48-1	✓	✓	-	✓	-	✓	✓
Chloroethane	75-00-3	✓	✓	-	✓	✓	✓	✓
2-Chloroethanol	107-07-3	*	-	-	-	-	-	✓

Compound	CAS No.[a]	5030	5035	5031	5032	5021	5041	Direct Inject
2-Chloroethyl vinyl ether	110-75-8	*	*	-	-	-	-	✓
Chloroform	67-66-3	✓	✓	-	✓	✓	✓	✓
1-Chlorohexane	544-10-5	✓	✓	-	-	-	-	-
Chloromethane	74-87-3	*	*	-	✓	✓	✓	✓
Chloroprene (2-Chloro-1,3-butadiene)	126-99-8	✓	-	-	-	-	-	✓
2-Chlorotoluene	95-49-8	✓	✓	-	✓	-	-	-
4-Chlorotoluene	106-43-4	✓	✓	-	✓	-	-	-
Crotonaldehyde	4170-30-3	*	-	*	-	-	-	✓
Cyclohexane	110-82-7	✓	✓	-	✓	-	-	-
1,2-Dibromo-3-chloropropane (DBCP)	96-12-8	*	*	-	✓	✓	-	✓
1,2-Dibromoethane (EDB, Ethylene dibromide)	106-93-4	✓	✓	-	✓	✓	-	✓
Dibromomethane	74-95-3	✓	✓	-	✓	✓	✓	✓
1,2-Dichlorobenzene	95-50-1	✓	✓	-	✓	✓	-	✓
1,3-Dichlorobenzene	541-73-1	✓	✓	-	✓	✓	-	✓
1,4-Dichlorobenzene	106-46-7	✓	✓	-	✓	✓	-	✓
cis-1,4-Dichloro-2-butene	1476-11-5	*	✓	-	✓	-	-	✓
trans-1,4-Dichloro-2-butene	110-57-6	*	✓	-	✓	-	-	✓
Dichlorodifluoromethane	75-71-8	*	*	-	*	✓	-	✓
1,1-Dichloroethane	75-34-3	✓	✓	-	✓	✓	✓	✓
1,2-Dichloroethane	107-06-2	✓	✓	-	✓	✓	✓	✓
1,1-Dichloroethene (Vinylidene chloride)	75-35-4	✓	✓	-	✓	✓	✓	✓
cis-1,2-Dichloroethene	156-59-2	✓	✓	-	✓	✓	-	-
trans-1,2-Dichloroethene	156-60-5	✓	✓	-	✓	✓	✓	✓
1,3-Dichloropropane	142-28-9	✓	✓	-	✓	-	-	-
1,2-Dichloropropane	78-87-5	✓	✓	-	✓	✓	✓	✓
2,2-Dichloropropane	594-20-7	✓	✓	-	✓	-	-	-
1,3-Dichloro-2-propanol	96-23-1	*	-	-	-	-	-	✓
1,1-Dichloropropene	563-58-6	✓	✓	-	✓	-	-	-
cis-1,3-Dichloropropene	10061-01-5	✓	✓	-	✓	-	✓	✓
trans-1,3-Dichloropropene	10061-02-6	✓	✓	-	✓	-	✓	✓
1,2,3,4-Diepoxybutane	1464-53-5	✓	-	-	-	-	-	✓
Diethyl ether	60-29-7	*	*	-	*	-	-	✓
Diisopropyl ether (DIPE)	108-20-3	*	✓	-	-	✓*	-	✓
1,4-Dioxane	123-91-1	*	*	✓	*	-	-	✓
Epichlorohydrin	106-89-8	*	*	-	-	-	-	✓
Ethanol	64-17-5	*	*	✓	*	✓*	-	✓
Ethyl acetate	141-78-6	*	*	✓	✓	-	-	✓
Ethyl benzene	100-41-4	✓	✓	-	✓	✓	✓	✓
Ethyl methacrylate	97-63-2	✓	✓	-	✓	-	-	✓
Ethyl *t*-butyl ether (ETBE)	637-92-3	✓*	✓*	-	-	✓*	-	✓
Ethylene oxide	75-21-8	*	-	✓	-	-	-	✓

Compound	CAS No.[a]	5030	5035	5031	5032	5021	5041	Direct Inject
Hexachlorobutadiene	87-68-3	*	✓	-	-	✓	-	✓
Hexachloroethane	67-72-1	*	*	-	✓	-	-	✓
2-Hexanone	591-78-6	*	*	-	✓	-	-	✓
Iodomethane (Methyl iodide)	74-88-4	✓	✓	-	✓	-	✓	✓
Isobutyl alcohol	78-83-1	*	*	✓	-	✓	-	✓
Isopropylbenzene	98-82-8	✓	✓	-	✓	✓	-	✓
p-Isopropyltoluene	99-87-6	✓	✓	-	✓	-	-	-
Malononitrile	109-77-3	*	-	-	-	-	-	✓
Methacrylonitrile	126-98-7	*	✓	✓	-	-	-	✓
Methanol	67-56-1	*	-	✓	-	-	-	✓
Methyl acetate	79-20-9	✓	✓	-	✓	-	-	-
Methyl acrylate	96-33-3	*	*	-	✓	-	-	-
Methyl methacrylate	80-62-6	✓	*	-	-	-	-	✓
Methyl tert-butyl ether (MTBE)	1634-04-4	✓*	✓*	-	✓	✓*	-	✓
Methylcyclohexane	108-87-2	✓	✓	-	✓	-	-	-
Methylene chloride (DCM)	75-09-2	✓	✓	-	✓	✓	✓	✓
4-Methyl-2-pentanone (MIBK)	108-10-1	*	*	✓	✓	-	-	✓
Naphthalene	91-20-3	*	*	-	✓	✓	-	✓
Nitrobenzene (NB)	98-95-3	✓	✓	-	-	-	-	✓
2-Nitropropane	79-46-9	✓	✓	-	-	-	-	✓
N-Nitroso-di-*n*-butylamine (*N*-Nitrosodibutylamine)	924-16-3	*	-	✓	-	-	-	✓
Paraldehyde	123-63-7	*	-	✓	-	-	-	✓
Pentachloroethane	76-01-7	*	*	-	*	-	-	✓
Pentafluorobenzene	363-72-4	✓	✓	-	✓	-	-	-
2-Pentanone	107-87-9	*	✓	✓	-	-	-	✓
2-Picoline (2-Methylpyridine)	109-06-8	*	*	✓	-	-	-	✓
1-Propanol (*n*-Propyl alcohol)	71-23-8	*	*	✓	-	-	-	✓
2-Propanol (Isopropyl alcohol)	67-63-0	*	*	✓	-	✓	-	✓
Propargyl alcohol	107-19-7	*	-	-	-	-	-	✓
β-Propiolactone	57-57-8	*	-	-	-	-	-	✓
Propionitrile (Ethyl cyanide)	107-12-0	✓	✓	✓	-	-	-	-
n-Propylamine	107-10-8	✓*	-	-	-	-	-	✓
n-Propylbenzene	103-65-1	✓	✓	-	✓	-	-	-
Pyridine	110-86-1	*	*	✓	*	-	-	✓
Styrene	100-42-5	*	*	-	✓	✓*	✓	✓
1,1,1,2-Tetrachloroethane	630-20-6	✓	✓	-	✓	✓	✓	✓
1,1,2,2-Tetrachloroethane	79-34-5	✓*	✓*	-	✓	✓*	✓	✓
Tetrachloroethene	127-18-4	✓*	✓	-	✓	✓*	✓	✓
Toluene	108-88-3	✓	✓	-	✓	✓	✓	✓
o-Toluidine	95-53-4	*	-	✓	-	-	-	✓
1,2,3-Trichlorobenzene	87-61-6	*	*	-	✓	✓	-	✓
1,2,4-Trichlorobenzene	120-82-1	*	*	-	✓	✓	-	✓
1,1,1-Trichloroethane	71-55-6	✓	✓	-	✓	✓	✓	✓

Compound	CAS No.[a]	5030	5035	5031	5032	5021	5041	Direct Inject
1,1,2-Trichloroethane	79-00-5	✓	✓	-	✓	✓	✓	✓
Trichloroethene (Trichloroethylene)	79-01-6	✓*	✓	-	✓	✓*	✓	✓
1,1,2-Trichloro trifluoroethane	76-13-1	✓	✓	-	✓	-	-	-
1,1,1-Trichlorotrifluoroethane	354-58-5	✓	✓	-	✓	-	-	-
Trichlorofluoromethane	75-69-4	*	*	-	✓	✓	✓	✓
1,2,3-Trichloropropane	96-18-4	✓	✓	-	✓	✓	✓	✓
1,2,3-Trimethylbenzene	526-73-8	-	-	-	-	✓	-	-
1,2,4-Trimethylbenzene	95-63-6	✓	✓	-	✓	✓	-	-
1,3,5-Trimethylbenzene	108-67-8	✓	✓	-	✓	✓	-	-
Vinyl acetate	108-05-4	*	*	-	-	-	-	✓
Vinyl chloride	75-01-4	*	*	-	✓	✓	✓	✓
m-Xylene	108-38-3	✓	✓	-	✓	✓	✓	✓
o-Xylene	95-47-6	✓	✓	-	✓	✓	✓	✓
p-Xylene	106-42-3	✓	✓	-	✓	✓	✓	✓

[a] Chemical Abstract Service Registry Number

KEY TO ANALYTE LIST

✓ Historically, adequate recovery and precision can be obtained for this analyte by this technique. However, actual recoveries may vary depending on the sample matrix, preparation technique, and analytical instrumentation. Data from a large multi-laboratory study for 5030 and 5035 is available in Table 2. Compounds with this flag had a relative standard deviation (RSD) ≤ 15% in a multi-laboratory study.

- Not determined

* This analyte exhibits known difficulties with reproducibility, response, recovery, stability, and/or chromatography that may reduce the overall quality or confidence in the result when using this preparation method combined with analysis by Method 8260 (e.g., multi-laboratory study data with a RSD >15%). This analyte may require special treatment (see Sec. 1.3) to improve performance to a level that would meet the needs of the project and, where necessary, may also require the use of appropriate data qualifiers if the relevant performance criteria cannot be met.

✓* This analyte meets the criteria for adequate performance using this technique (see definition for ✓); however, it is known to exhibit problems listed in Sec. 1.3 (see definition for *).

1.2 The compounds listed above may be introduced into the gas chromatograph/mass spectrometer (GC/MS) system by various techniques. The techniques listed in the table above have performance data available. Purge-and-trap, by Methods 5030 (aqueous samples) and 5035 (solid and waste oil samples), is the most commonly used technique for VOCs. However, other techniques are also appropriate and may yield better performance for some analytes.

These include: direct injection after dilution with hexadecane (Method 3585) for waste oil samples; automated static headspace by Method 5021 for solid and aqueous samples; direct injection of an aqueous sample (concentration permitting) or injection of a sample concentrated by azeotropic distillation (Method 5031); and vacuum distillation (Method 5032) for aqueous,

solid, oil and tissue samples. For air samples, Method 5041 provides methodology for desorbing VOCs from trapping media (Methods 0010, 0030, and 0031). In addition, direct analysis utilizing a sample loop is used for sub-sampling from polytetrafluoroethylene (PTFE) bags (Method 0040), also referred to as Tedlar® bags. Method 5000 provides more general information on the selection of the appropriate introduction method.

1.3 Special considerations for compounds noted with * in the table in Sec. 1.1.

1.3.1 Recovery of bases from water will be affected by pH. Compounds such as pyridine, o-toluidine, n-propylamine and 2-picoline will have poor to no recovery from low pH water.

1.3.1.1 2-Chloroethyl vinyl ether is subject to hydrolysis at low pH.

1.3.2 Dehydrohalogenation may result in degradation of aqueous solutions of pentachloroethane and to a lesser extent, other halogenated compounds (e.g., dichlorobutenes and 1,1,2,2-tetrachloroethane) to other target analytes (especially tetrachloroethene and trichloroethene) if the pH is >4 (see Reference 6 in Sec. 16 for further information on this topic). The use of hydrogen carrier gas may also cause the dehydrohalogenation of these analytes.

1.3.3 Alcohols, ketones, ethers and other water-soluble compounds will have low responses. Elevated sample equilibration temperatures may be necessary during preparation. Elevated sample temperatures may be necessary during purges as heated samples will exhibit better performance of these analytes. However, ethers such as diethyl ether and MTBE hydrolyze more readily when heated in acid-preserved water. Acid preservation is not recommended for analysis of these target analytes at elevated sample temperature. Higher concentrations for calibration standards may also be appropriate. Methanol is used as a solvent for standards in this analysis. Therefore, special conditions and alternate standards will be required for analyses where it is a target analyte.

1.3.4 Aldehydes (e.g., acrolein, paraldehyde, crotonaldehyde) are included in the target list but have poor stability under the analytical conditions used in this method. Other methods may be more appropriate for these compounds.

1.3.5 Heavier target compounds (e.g., naphthalene, 1,2-dibromo-3-chloropropane and hexachlorobutadiene) will have lower overall response and greater variability with conditions and concentrations.

1.3.6 Compounds that are gases at room temperature (e.g., chlorofluorocarbons, chloromethane and vinyl chloride) are prone to loss through vial seals and in handling. In addition, compounds co-eluting with water and methanol will have their responses suppressed.

1.3.7 Vinyl chloride and styrene are subject to loss due to chemical reactivity. Preservation by acidification does not prevent this.

1.4 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 5000 and 8000) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the SW-846 manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, supplies, and on

the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by the Environmental Protection Agency (EPA or the Agency) as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.5 This method is restricted to use by, or under supervision of, personnel appropriately experienced and trained in the use of GC/MS and skilled in the interpretation of mass spectra. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 VOCs are introduced into the GC by one of the preparation methods mentioned in Secs. 1.1 and 1.2. The analytes may be introduced directly to a capillary column, cryofocused on a capillary pre-column before being flash evaporated to a capillary column for analysis, or desorbed from a trap and sent to an injection port operating in the split mode for injection to a capillary column. The column is temperature-programmed to separate the analytes, which are then detected with a MS interfaced to the GC.

2.2 Analytes eluted from the capillary column are introduced into the MS via a direct connection or flow splitter. Some wide-bore capillary columns may require splitting the flow prior to the MS interface, whereas narrow-bore capillary columns may be directly interfaced to the ion source or used with a restrictor column at the MS interface. Qualitative identification of target analytes is accomplished by comparing their mass spectra and retention times (RTs) with the mass spectra and RTs of known standards for the target compounds. Quantitation is accomplished by comparing the response of a major (quantitation) ion relative to an internal standard (IS) using an appropriate calibration curve for the intended application.

2.3 The method includes specific calibration and QC steps that supersede the general requirements provided in Method 8000.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 In order to avoid compromising data quality, contamination of the analytical system by volatile materials from the laboratory must be reduced to the lowest practical level. Refer to each preparation method for specific guidance on QC procedures and to Chapter Four for general guidance on the cleaning of glassware. Refer to Method 8000 for a discussion of interferences.

4.2 Volatile preparation and analysis should be physically separated from laboratory areas where target solvents are used. Air supply for the volatiles area should provide positive

pressure relative to other laboratory areas. The water supply used for blanks should be isolated from target solvents and free of plastic supply piping.

4.3 Cross contamination may occur when a sample containing low concentrations of VOCs is analyzed immediately after a sample containing high concentrations of VOCs. After analysis of a sample containing high concentrations of VOCs, analysis of one or more blanks may be used to demonstrate that carryover is not a significant portion of the target response in subsequent samples.

4.4 For samples that may contain large amounts of surfactants, suspended solids, high boiling compounds, high concentrations of target analytes or other non-target interferences, screening samples with another technique prior to purge-and-trap GC/MS analysis is prudent to prevent system contamination.

4.5 Control of contaminants is assessed by analysis of blanks. Transport (trip), calibration and reagent blanks provide information about the presence of contaminants at different points in the analytical process. Where measured analyte concentrations are suspected of being biased high or having false positive results due to contamination, affected data should be qualified, and the data user should otherwise be informed of any suspected data quality issues. Subtracting blank values from sample results is not permitted.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and maintaining a current awareness of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals listed in this method. A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses. If hydrogen is used as a carrier gas, see Appendix B.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

6.1 This section does not list common laboratory glassware (e.g., beakers and flasks).

6.1.1 Purge-and-trap device for aqueous samples as described in Method 5030

6.1.2 Purge-and-trap device for solid samples as described in Method 5035

6.1.3 Automated static headspace device for solid and aqueous samples as described in Method 5021

6.1.4 Azeotropic distillation apparatus for aqueous and solid samples as described in Method 5031

6.1.5 Vacuum distillation apparatus for aqueous, solid and tissue samples as described in Method 5032

6.1.6 Desorption device for air trapping media for air samples as described in Method 5041

6.1.7 Air sampling loop for sampling from Tedlar® bags for air samples as described in Method 0040

6.2 GC/MS system

6.2.1 GC – An analytical system complete with a temperature-programmable GC suitable for splitless injection with an appropriate interface or direct split interface for sample introduction. The system includes all required accessories, including syringes, analytical columns, and gases. If hydrogen is used as a carrier gas, see Appendix B.

6.2.1.1 The GC should be equipped with flow controllers such that the column flow rate remains constant throughout desorption and temperature program operation.

6.2.1.2 For some column configurations, the column oven must be cooled to less than 30 °C. Therefore, a sub-ambient oven controller may be necessary.

6.2.1.3 A capillary column can be directly coupled to the ion source of the MS or interfaced through a separator, depending on the size of the capillary and the requirements of the GC/MS system.

6.2.1.4 GC columns – The following columns have been found to provide good separation of VOCs:

- 30 m x 0.25 mm internal diameter (ID), 1.4-µm film thickness, DB-624 or VOCOL;
- 20 m x 0.18 mm ID, 1-µm film thickness, DB-VRX;
- 60 m x 0.32 mm ID, 1.5-µm or 1.8-µm film thickness, Rtx-Volatiles.

The following columns were used to generate performance data cited in the references:

- 30 m x 0.25 - 0.32 mm ID, 1-µm film thickness, DB-5, Rtx-5, SPB-5; and
- 75 m x 0.53 mm ID, 3-µm film thickness, DB-624, Rtx-502.2, or VOCOL.

6.2.2 MS

6.2.2.1 The MS must be capable of acquiring mass spectra from mass/charge (*m/z)* 35 to 270 at a rate fast enough to acquire at least five (but preferably 10 or more) mass spectra across each chromatographic peak of interest, using 70 volts (nominal) electron energy in the electron impact ionization mode. The MS must be also capable of meeting the criteria as outlined in Sec. 11.3.1.

6.2.2.2 An ion trap MS may be used if it is capable of axial modulation to reduce ion-molecule reactions and can produce electron impact-like spectra that match those in the EPA/National Institute on Standards and Technology (NIST) library or equivalent. Because ion-molecule reactions with water and methanol in an ion trap MS may produce interferences that co-elute with

chloromethane and chloroethane, the base peak for both of these analytes will be at *m/z* 49, which should also be used as the quantitation ion in this case. The MS must be capable of producing a mass spectrum which meets the criteria as outlined in Sec. 11.3.1.

6.2.2.3 A tandem MS (MS/MS) may be used if it has the necessary pumps, collision cell, collision gases, and high-vacuum system capable of performing transitions in product ion scan mode or the selected reaction monitoring mode (SRM) for the target analytes of interest. Recommendations for specific precursor and product ions in SRM are available for some target analytes from the manufacturers of the equipment. The system must be capable of documenting the performance of both MSs against manufacturer specifications for mass resolution, mass assignment, and sensitivity using the internal calibrant (e.g., perfluorotributylamine). It is recommended to check the performance of the system at least weekly or at a frequency appropriate to meet the needs of the project. At a minimum, the performance of the system must be checked just prior to the initial calibration (ICAL).

6.2.2.4 The use of selected ion monitoring (SIM) or chemical ionization (CI) mass spectrometry are acceptable techniques for applications requiring quantitation limits below the normal range of electron impact mass spectrometry or to reduce interferences from the sample matrix.

6.2.3 GC/MS interface – One of the following examples may be used to interface the GC to the MS.

6.2.3.1 Direct coupling, by inserting the column into the MS through a heated transfer line, is generally used for capillary columns <0.53 mm ID.

6.2.3.2 A jet separator, including an all-glass transfer line and glass enrichment device or split interface, is used with columns ≥0.53 mm ID.

6.2.3.3 Other interfaces may be used provided the performance specifications described in Sec. 11.3.1 are achieved.

6.2.4 Data system – A computer system (that allows the continuous acquisition and storage of all mass spectra obtained throughout the duration of the chromatographic program) must be interfaced to the MS. The computer must have software that allows searching any GC/MS data file for ions of a specified mass and plotting such ion abundances versus time or scan number. This type of plot is defined as an extracted ion current profile (EICP). Software must also be available that allows integrating the abundances in any EICP between specified time or scan-number limits. A recent version of the EPA/NIST mass spectral library, or equivalent, should also be available.

6.3 Microsyringes – 10, 25, 100, 250, 500, and 1000 µL gas-tight

6.4 Syringe valve – Two-way, with Luer ends (three each), if applicable to the purging device

6.5 Syringes – 5, 10, or 25 mL, gas-tight with shutoff valve

6.6 Balance – Analytical, capable of weighing 0.0001 g, and top-loading, capable of weighing 0.1 g

6.7 Glass VOA vials – 20, 40, 60 mL, with PTFE-lined screw-top or crimp-top caps (compatible with the autosampler if appropriate for the preparation technique)

6.8 Vials – for GC autosampler

6.9 Disposable pipets – Pasteur

6.10 Volumetric flasks, Class A – 5, 10, 50, 100 mL, with ground-glass stoppers

6.11 Spatula – Stainless steel

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available at: http://pubs.acs.org/reagents/comminfo/techquestions.html. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Organic-free reagent water – All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

7.3 Methanol, CH_3OH – Purge-and-trap grade or equivalent, demonstrated to be free from interferences for the compounds of interest at their lower limit of quantitation (LLOQ). Store this solvent apart from other solvents to avoid contamination.

7.4 Hexadecane – Reagent grade, or equivalent, demonstrated to be free from interferences for the compounds of interest at the levels of interest through the analysis of a solvent blank. The results of such a blank analysis must demonstrate that no interfering volatiles are present.

7.5 1:1 Volume/volume (v/v) hydrochloric acid (HCl/water) – Carefully add a measured volume of concentrated HCl to an equal volume of organic-free reagent water.

7.6 Stock standard solutions – The solutions may be purchased as certified solutions or prepared from pure standard materials. Commercially prepared stock standards may be used at any concentration if they are certified by an accredited supplier or third party. Prepare stock standard solutions in methanol (or other appropriate solvent), using assayed liquids or gases, as appropriate.

7.6.1 Certified solutions purchased from a vendor must be replaced per the manufacturer's recommended expiration date. Stock standard solutions prepared in-house must be replaced after one year, or sooner if comparison with QC check samples indicates a problem. When solutions are mixed together, regardless of the source, they must be replaced after the manufacturer's expiration date or one year (whichever occurs first) or sooner if problems are indicated. The assigned expiration date of the mixed standard should correspond to that of the stock that expires the earliest.

7.7 Working standards – Using stock standard solutions, prepare working standards in methanol (or other appropriate solvent), containing the compounds of interest, either singly or mixed together. Working standards should be stored with minimal headspace and should be

checked frequently for signs of degradation or evaporation, especially just prior to preparing calibration standards. Working standards for most compounds should be replaced after four weeks unless the integrity of the standard is suspected of being compromised prior to that time. Working standards for gases should be replaced after one week unless the acceptability of the standard can be documented. When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

7.8 Surrogate standards – Recommended general-use surrogates are toluene-d_8, 4-bromofluorobenzene (BFB), and 1,2-dichloroethane-d_4. Other compounds with physicochemical properties better resembling the analyte classes of interest may be used as surrogates (e.g., deuterated monitoring compounds in the EPA Contract Laboratory Program's (CLP) current statement of work, which can be found in Reference 14 in Sec. 16), provided they can be unambiguously identified and meet any applicable acceptance criteria described in Sec. 11 for ICAL and continuing calibration verification (CCV). A stock surrogate solution should first be prepared in methanol, and a surrogate standard spiking solution should then be prepared from the stock at an appropriate concentration in methanol. Each sample undergoing GC/MS analysis must be spiked with the surrogate spiking solution prior to analysis.

7.9 Internal standards (IS) – The recommended ISs are fluorobenzene, chlorobenzene-d_5, and 1,4-dichlorobenzene-d_4. Other compounds may be used as ISs as long as they have RTs similar to their target compounds, they can be unambiguously identified, and meet any applicable acceptance criteria described in Sec. 11. See Sec. 11.4.3 of Method 8000 for additional information. Prepare the ISs solution in methanol (or other appropriate solvent).

7.10 4-Bromofluorobenzene (BFB) tune verification standard – A standard solution of BFB in methanol (or other appropriate solvent) may be prepared for direct injection. If BFB is used as a surrogate, the surrogate solution may be used for this purpose.

7.11 Calibration standards – There are two types of calibration standards used for this method: standards made from the primary source (for ICAL and CCV) and standards made from a second source for initial calibration verification (ICV). When using premixed certified solutions, store them according to the manufacturer's documented holding time and storage temperature recommendations.

7.11.1 ICAL standards must be prepared at a minimum of five different concentrations from the working dilution of stock standards or from premixed certified solutions. Prepare these solutions in organic-free reagent water or in a solvent appropriate for the specific sample preparation method used. Include a minimum of five different concentrations in the calibration for average response factor (RF) or linear (first-order) calibration models or six different concentrations for a quadratic (second-order) model, with the low standard at or below the LLOQ (see Sec. 9.9 and Method 8000). At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the DQOs of the project. The remaining standards should correspond to the range of concentrations found in typical samples but should not exceed the working range of the GC/MS. ICAL standards should be mixed from fresh stock standards and dilution standards when generating an ICAL curve.

7.11.2 CCV standards should be prepared at a concentration near the mid-point of the ICAL from the same source as the ICAL.

7.11.3 Second source standards for ICV must be prepared using source materials from a second manufacturer or from a manufacturer's batch prepared independently from the batch used for calibration. A second lot number from the same manufacturer may be adequate to meet this requirement. Target analytes in the ICV

are recommended to be prepared at concentrations near the mid-point of the calibration range. The standard must contain all calibrated target analytes that will be reported for the project, if readily available. See Secs. 9.3.2 and 11.3.6 for guidance and acceptance limits.

7.11.4 It is the intent of EPA that all target analytes for a particular analysis be included in the ICAL and CCV standard(s). These target analytes may not include the entire list of analytes (Sec. 1.1) for which the method has been demonstrated. However, the laboratory shall not report a quantitative result for a target analyte that was not included in the calibration standards.

7.12 Matrix spikes and Laboratory Control Samples (LCS) – See Method 5000 for instructions on preparing the matrix spike standard. Matrix spikes and LCSs should be prepared with target analytes from the same source as the ICAL standards to restrict the influence of accuracy on the determination of recovery throughout preparation and analysis. Add VOCs to matrix spikes and LCS standards that are representative of the compounds being investigated. It is recommended that all target analytes being investigated be included in all LCS and matrix spiked samples. For some applications, a limited set of representative analytes is acceptable.

7.13 Great care must be taken to maintain the integrity of all standard solutions. It is recommended that standards be stored with minimal headspace, protected from light, at ≤6 °C, or as recommended by the standard manufacturer using screw-cap or crimp-top amber containers equipped with PTFE liners. Returning standards to the refrigerator or freezer immediately after standard and sample preparation is completed will help maintain the integrity of the solutions and minimize loss of volatile target compounds. IS and surrogate spiking solutions added by the instrument do not need to be refrigerated provided they are sealed to prevent loss.

7.14 Carrier gas – Helium or hydrogen may be used as a carrier gas. If hydrogen is used, analytical conditions may need to be adjusted for optimum performance and calibration, and all QC tests must be performed with hydrogen carrier gas. See Appendix B for guidance.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in a regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

8.1 See Chapter Four, "Organic Analytes", for storage condition and holding times.

8.2 Aqueous samples should be stored with minimal or no headspace to minimize the loss of highly volatile analytes.

8.3 Solid and waste samples should be collected in air-tight containers compatible with closed-system sample preparation and analysis techniques, if possible. Samples must be handled carefully to minimize loss of VOCs during sample collection, shipping, storage, preparation and analysis. Refer to Chapter 4 and to American Society for Testing and Materials (ASTM) D4547 (Reference 18) for more information.

8.4 Samples to be analyzed for VOCs should be stored separately from standards and from other samples expected to contain significantly different concentrations of volatile compounds, or from samples collected for the analysis of other parameters such as semivolatile organic chemicals.

8.5 Blanks should be used to monitor potential cross-contamination of samples due to improper handling or storage conditions. The specifics of this type of monitoring activity should be outlined in a laboratory SOP or project planning documents pertaining to volatiles sampling.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a quality assurance project plan (QAPP) or a sampling and analysis plan (SAP), which translates project objectives and specifications into directions for those who will implement the project and assess the results. Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for general QC procedures for organic determinative methods. Refer to Method 5000 for QC procedures to ensure the proper operation of the various sample preparation techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 8000 and 5000.

9.3 QC procedures necessary to evaluate GC system operation are found in Method 8000 and include evaluation of RT windows, calibration verification and chromatographic analysis of samples. In addition, discussions regarding the instrument QC categories, minimum frequency and criteria listed below can be found in the referenced sections of this method, and a summary is provided in Table 7. Quantitative sample analyses should not proceed for those analytes that do not meet the QC acceptance criteria. However, analyses may continue for those analytes that do not meet the criteria with an understanding that these results could be used for screening purposes and would be considered estimated values.

9.3.1 The GC/MS tune must be verified to meet acceptance criteria prior to ICAL. Acceptance criteria are primarily intended to verify mass assignments and mass resolution under the same conditions used for analysis (refer to Sec. 11.3.1)

9.3.2 There must be an ICAL of the GC/MS system as described in Sec. 11.3. Prior to analyzing samples, the ICAL must be verified using a second source ICV standard, if readily available (refer to Sec. 11.3.6).

9.3.3 Calibration of the system must be verified periodically by analysis of a CCV standard. See Sec. 11.4 for the frequency and acceptance criteria.

9.4 Initial demonstration of proficiency (IDP) - Prior to implementation of a method, each laboratory must perform an IDP consisting of at least four replicate reference samples spiked into a clean matrix taken through the entire sample preparation and analysis. If an autosampler is used to make sample dilutions, the accuracy of the dilutions should be evaluated

prior to sample analysis. Whenever a significant change to instrumentation or procedure occurs, the laboratory must demonstrate that acceptable precision and bias can still be obtained. Also, whenever new staff members are trained, each analyst must perform an IDP for the method or portion of the method for which the analyst is responsible. This demonstration should document that the new analyst is capable of successfully following the SOP established by the laboratory and meeting any applicable acceptance criteria specified therein. Refer to Sec. 9.3 of Method 8000 for more information on how to perform an IDP.

9.5 Blanks

9.5.1 Before processing any samples, the analyst must demonstrate through the analysis of a method blank (MB) or instrument blank that equipment and reagents are free from contaminants and interferences. If a peak is found in the blank that would prevent the identification or bias the measurement of an analyte, the analyst should determine the source and eliminate it, if possible. As a continuing check, each time a batch of samples is analyzed, and when there is a change in reagents, a MB must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. MBs, trip blanks, and other field blanks must be carried through all stages of sample preparation and analysis. At least one MB must be analyzed on every instrument after calibration standard(s) and prior to the analysis of any samples. Blank(s) analyzed after a high concentration calibration standard can also be used to estimate the extent of decontamination needed to reduce the signal to an acceptable level (Sec. 9.5.2) after analyzing a sample at a similar concentration.

9.5.2 Blanks are generally considered to be acceptable if target analyte concentrations are less than one half the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., target analytes are not present in samples or sample concentrations/responses are >10X the blank). The analyst (or laboratory) should document detected common laboratory contaminants and distinguish those from situations (e.g., carryover), where corrective action may be required. Other criteria may be used depending on the needs of the project.

9.5.3 If an analyte of interest is found in a sample in the batch near a concentration detected in the blank (refer to Sec. 9.5.2), the presence and/or concentration of that analyte should be considered suspect and may require qualification. Contaminants in the blank should meet most or all of the qualitative identifiers in Sec. 11.6 to be considered a valid detection. Samples may require re-analysis if the blanks do not meet laboratory-established or project-specific criteria. Re-analysis is not necessary if the analyte concentration falls well below the action or regulatory limit or if the analyte is deemed not important for the project.

9.5.4 When new reagents or chemicals are received, the laboratory should monitor the blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks should be prepared for each set of reagents.

9.5.5 The laboratory should not subtract the results of the MB (or any blank) from those of any associated samples. Such "blank subtraction" may lead to negative sample results. If the MB results do not meet project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the MB results, and a discussion of the corrective actions undertaken by the laboratory.

9.6 Sample QC for preparation and analysis – The laboratory must also have procedures for documenting the effect of the sample matrix on method performance (i.e., precision, bias, and method sensitivity). At a minimum, this must include the analysis of a MB and LCS, and, where practical, either a laboratory sample duplicate/matrix spike or matrix spike/matrix spike duplicate (provided sufficient material is made available to the laboratory for doing so) in each preparation batch of 20 or fewer samples, as well as monitoring the recovery of surrogates in all samples. These QC samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on the field samples.

9.6.1 A MB must be included with each preparation batch. MBs consist of an aliquot of clean (control) matrix similar to the sample and of a similar weight or volume. Other types of blanks (e.g., trip blanks, storage blanks, etc.) should be included when appropriate but are distinct from MBs.

9.6.2 An LCS must be included with each preparation batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike, when appropriate. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. The LCS for water sample matrices is typically prepared in organic-free reagent water similar to the CCV standard. The LCS for solid matrices may also include clean sand, but the use of sand is not required. When an LCS is prepared in the same manner as a CCV, the same standard can be used as both the LCS and CCV. The CCV acceptance criteria may be used for evaluation in this situation. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 Documenting the effect of the matrix on target analyte measurements should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision of whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on knowledge of the samples, and project goals. These should be addressed in the project planning documents. If samples are expected to contain reportable levels of target analytes, then laboratories may use one matrix spike and a duplicate analysis of a non-spiked field sample. If samples are not expected to contain reportable levels of target analytes, laboratories may use a matrix spike and matrix spike duplicate pair. Consult Method 8000 for information on developing acceptance criteria for the matrix spike/laboratory sample duplicates or matrix spike/matrix spike duplicates. When spiking solid samples in an aqueous mixture, it is not practical to expect analyte behavior equivalent to an exposure that occurred in field conditions. Therefore, it is understood that matrix spikes are used to estimate the severity of matrix effects that can be observed within method constraints.

9.6.4 See Method 8000 for more details on carrying out QC procedures for preparation and analysis. In-house criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.7 Surrogate recoveries – Surrogates must be added to every blank, field sample, laboratory QC, and field QC. The laboratory should evaluate surrogate recovery data from individual samples relative to the surrogate recovery acceptance criteria developed by the laboratory. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate recovery acceptance criteria. Suggested surrogate recovery limits for field samples are 70 to 130% until laboratory or project-specific criteria can be developed. Limits will depend on the surrogates chosen, levels used, and instrument conditions. Procedures for

evaluating the recoveries of multiple surrogates and associated corrective actions should be defined in the laboratory's SOP or in an approved project plan.

9.8 IS responses must be monitored to ensure sensitivity is maintained and to limit the potential for measurement bias of associated target analyte concentrations. IS responses in field samples are compared to responses of the same ISs in the ICAL standards or CCV standards, with suggested acceptance criteria provided in Sec. 11.5.6. When IS responses fall outside the acceptance range, further investigation is warranted and results may require qualification for detects and non-detects.

9.9. Lower limit of quantitation (LLOQ) – The LLOQ is the lowest concentration at which the laboratory has demonstrated target analytes can be reliably measured and reported with a certain degree of confidence, which must be greater than or equal to the lowest point in the calibration curve. The laboratory shall establish the LLOQ at concentrations where both quantitative and qualitative criteria can consistently be met (see Sec. 11.6). The laboratory shall verify the LLOQ at least annually and whenever significant changes are made to the preparation and/or analytical procedure, to demonstrate quantitation capability at lower analyte concentration levels. The verification is performed by the preparation and/or analysis of an LCS (or matrix spike) at 0.5 - 2 times the established LLOQ. Additional LLOQ verification may be useful on a project-specific basis if a matrix is expected to contain significant interferences at the LLOQ. This verification may be accomplished with either clean control material (e.g., reagent water, solvent blank, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix, free of target compounds. Optimally, the LLOQ should be less than the desired decision level or regulatory action level based on the stated DQOs.

9.9.1 LLOQ verification

9.9.1.1 The verification of LLOQs using spiked clean control material represents a best-case scenario because it does not evaluate the potential matrix effects of real-world samples. For the application of LLOQs on a project-specific basis, with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.9.1.2 The LLOQ verification is prepared by spiking a clean control material with the analyte(s) of interest at 0.5 - 2 times the LLOQ concentration level(s). Alternatively, a representative sample matrix free of targets may be spiked with the analytes of interest at 0.5 - 2 times the LLOQ concentration levels. This LLOQ check is carried through the same preparation and analytical procedures as environmental samples and other QC samples. LLOQ verification samples must be independent from the ICAL used to calculate the target analyte concentrations (i.e., not a recalculated calibration point). It is recommended that the LLOQ be to verified on every instrument where data is reported. However, at a minimum, the laboratory should rotate the verification among similar analytical instruments such that all are included within three years.

9.9.1.3 Recovery of target analytes in the LLOQ verification should be within established in-house limits or within other such project-specific acceptance limits to demonstrate acceptable method performance at the LLOQ. Until the laboratory has sufficient data to determine acceptance limits, the LCS criteria of ±20% (i.e., lower limit minus 20% and upper limit plus 20%) may be used for the LLOQ acceptance criteria. This practice acknowledges the potential for greater uncertainty at the low end of the calibration curve. Practical, historically based LLOQ acceptance criteria should be determined once sufficient data points have been acquired.

9.9.2 Reporting concentrations below LLOQ – Concentrations that are below the established LLOQ may still be reported. However, these analytes must be qualified as estimated. The procedure for reporting analytes below the LLOQ should be documented in the laboratory's SOP or in a project-specific plan. Analytes below the LLOQ that are reported should meet most or all of the qualitative identification criteria in Sec. 11.6.

9.10 It is recommended that the laboratory adopt additional QA practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Secs. 11.3 and 11.4 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Various alternative methods are provided for sample introduction. All ISs, surrogates, and matrix spike compounds (when applicable) must be added to the samples before introduction into the GC/MS system. Consult the sample introduction method for the procedures by which to add such standards.

11.1.1 Direct injection – This includes: injection of an aqueous sample containing a very high concentration of analytes; injection of aqueous concentrates from Method 5031 (azeotropic distillation); and injection of a waste oil diluted with hexadecane (Method 3585). Direct injection of aqueous samples (non-concentrated) has very limited applications. Direct injection of aqueous samples is only used for the determination of volatiles at the toxicity characteristic (TC) regulatory limits or at mg/L or higher concentrations. Direct injection may also be used in conjunction with the test for ignitability in aqueous samples (along with Methods 1010 and 1020), to determine if alcohol is present at greater than 24%.

11.1.2 Purge and trap – This includes purge and trap for aqueous samples (Method 5030) and purge and trap for solid samples (Method 5035). Method 5035 also provides techniques for extraction of high concentration solid and oily waste samples by methanol (and other water-miscible solvents) with subsequent purge and trap from an aqueous matrix using Method 5030.

11.1.2.1 Traditionally, the purge and trap of aqueous samples is performed at ambient temperature, while purging of soil/solid samples is performed at 40 °C, to improve purging efficiency. Purging aqueous samples at a fixed temperature slightly above ambient (e.g., 35 °C) may improve reproducibility where ambient temperature is variable.

11.1.2.2 Aqueous and soil/solid samples may also be purged at higher temperatures as long as all calibration standards, field samples, and associated QC samples are purged at the same temperature, and the laboratory demonstrates acceptable method performance for the project. Purging of aqueous and soil/solid samples at elevated temperatures (i.e., 40 to 80 °C) may improve the purging performance of more highly water-soluble compounds which

have poor purging efficiencies at ambient temperatures.

11.1.3 Vacuum distillation – This technique may be used for the introduction of VOCs from aqueous, solid, or tissue samples (Method 5032) into the GC/MS system (see Method 8261).

11.1.4 Automated static headspace – This technique may be used for the introduction of VOCs from aqueous and solid samples (Method 5021) into the GC/MS system.

11.1.5 Cartridge desorption – This technique may be used for the introduction of VOCs from sorbent cartridges (Method 5041) used in the sampling of air. The sorbent cartridges are from the volatile organics sampling train (VOST) or sampling method for volatile organic compounds (SMVOC) (Method 0031).

11.2 Recommended chromatographic conditions are provided as examples based on analyses performed in EPA laboratories and studies used to generate performance data for this method. The actual conditions will depend on the compounds of interest, instrument, and manufacturer's guidelines for the column selected. The maximum temperature of operation should always be verified with the specific column manufacturer.

11.2.1 General conditions:

Injector temperature:	200 - 275 °C
Transfer line temperature:	200 - 300 °C

11.2.2 Direct split interface – The following are example conditions:

Carrier gas (He) flow rate:	1.3 mL/min
Column:	60 m x 0.25 mm ID, 1.4 µm DB-624
Initial temperature:	35 °C, hold for 3 min
Temperature program:	6 °C /min to 100 °C, 12 °C /min to 180 °C, 20 °C /min to 200 °C, hold for 7 minutes
Inlet temperature:	225 °C
Transfer line temperature:	230 °C
Split ratio:	30:1

11.2.3 Split injection:

Carrier gas (He) flow rate:	0.9 mL/min
Column:	20.0 m, 0.18 mm ID, 1.0 µm DB-VRX
Initial temperature:	30 °C, hold for 3 min
Temperature program:	10 °C /min to 100 °C, 20 °C /min to 240 °C; 1 minute hold
Inlet temperature:	250 °C
Transfer line temperature:	250 °C
Split ratio:	50:1

11.2.4 Split injection:

Carrier gas (He) flow rate:	0.7 mL/min
Column:	20 m x 0.18 mm x 1.0 µm DB-624
Initial temperature:	40 °C, hold for 4 min
Temperature program:	15 °C /min to 190 °C,

Carrier gas (He) flow rate:	0.7 mL/min hold for 1.5 min at 250 °C
Split ratio:	35:1

11.2.5 Direct injection:

Carrier gas (He) flow rate:	4 mL/min
Column:	70 m x 0.53 mm DB-624
Initial temperature:	40 °C, hold for 3 min
Temperature program:	8 °C /min to 260 °C

11.2.6 Hydrogen carrier gas:

Flow rate:	1 mL/min
Column:	40 m x 0.18 mm x 1-µm film thickness Rtx-VMS
Initial temperature:	30 °C, hold for 4 min
Temperature program:	7 °C /min to 180 °C
Injector temperature:	200 °C
Transfer line temperature:	200 °C
Split ratio:	70:1

11.3 ICAL – Establish the GC/MS operating conditions, using the following as guidance:

Mass range:	*m/z* of 35 – 270
Acquisition rate:	To result in at least five mass spectra across the peak (but preferably ten or more)
Source temperature:	According to manufacturer's specifications
Ion trap only:	Set axial modulation, manifold temperature, and emission current to manufacturer's recommendations

11.3.1 The GC/MS system must produce mass spectra with sufficient mass accuracy, mass resolution, and signal to be used for quantitative analysis of specific *m/z* ratios of ions characteristic of the target analytes, surrogates, and ISs. Standardization of MS performance also simplifies comparison of mass spectra generated on different instruments, such as by searching unknown spectra against a commercially available mass spectral library. A common reference compound used to demonstrate MS performance for electron impact mass spectrometry is BFB. Table 3 provides BFB ion ratio evaluation criteria. These criteria are only appropriate for electron impact mass spectra acquired across the range of masses indicated in the table.

Acceptable system performance may also be demonstrated by meeting manufacturer specifications for mass resolution, mass accuracy, and sensitivity using the internal calibrant (e.g., perfluorotributylamine, also known as PFTBA). Other reference compounds may also be appropriate for demonstrating acceptable MS performance depending on the system or conditions used for analysis (e.g., octafluoronaphthalene for negative ion CI). Regardless of how MS performance is evaluated, system calibration must not begin until performance criteria are met, and calibration standards and samples must be analyzed under the same conditions, *i.e.*, if the system is retuned a new calibration should be performed. If CI, SIM or tandem MS is used, the manufacturer's MS tuning criteria or one of the alternative procedures listed above may be substituted for the BFB tune verification requirement.

11.3.1.1 In the absence of other recommendations on how to acquire

the mass spectrum of BFB, the following approach may be used:

Introduce BFB with the same technique to be used for analysis of calibration standards and samples. Scale the mass of BFB introduced to prevent high abundance masses from saturating the detector (e.g., ≤50 ng). Once the data is acquired, either select the mass spectrum at the peak apex for evaluation, or use an averaged mass spectrum (e.g., three highest abundance spectra, or across entire BFB peak). Background subtraction is allowed and should only be used to eliminate column bleed or instrument background ions. No part of the BFB peak or any other discrete peak should be subtracted. The mass spectrum used for background subtraction may be either a single mass spectrum or an average mass spectrum across a short time range acquired within 20 seconds of the elution of BFB.

11.3.1.2 Compare BFB mass intensities to the criteria in Table 3. Alternatively, other documented ion ratio criteria may be used provided that method performance is not adversely affected. If hydrogen is used as a carrier gas, the Table 3 criterion for 96/95 *m/z* ratio of BFB will be difficult to achieve. A relative abundance of 5 to 15% for 96/95 *m/z* is acceptable due to interactions with the carrier gas and water vapor. The analyst is free to choose criteria that are tighter than those included in this method or to use other documented criteria provided they are used consistently throughout the ICAL, calibration verification, and sample analyses.

NOTE: All subsequent standards, field samples, and QC samples associated with this analysis must use identical MS instrument conditions with the exception of SIM analysis. BFB may be analyzed in full scan mode while standards, samples, and QC are analyzed in SIM. As an alternative to BFB for SIM analysis, the laboratory may also use an alternative detector verification, such as PFTBA, or the manufacturer's recommended detector check.

NOTE: BFB tune checks are not appropriate for CI or tandem MS analysis using SRM. However, the laboratory must demonstrate, prior to the ICAL, that the MS system achieves mass accuracy and mass resolution criteria specified by the instrument manufacturer for the PFTBA internal calibrant or another appropriate chemical.

11.3.2 Set up the sample introduction system, and then prepare and analyze calibration standards as outlined in the preparation method of choice (see Sec. 11.1). ICAL standards must include at least five different standard concentrations for all target analytes (see Sec. 7.11.1 and Method 8000). Surrogates may be calibrated either at multiple concentrations in the ICAL or at a single concentration (i.e., constant amount added to each calibration standard, as with IS). The base peak *m/z* of each target analyte and IS is appropriate for use as the primary *m/z* for quantitation (see Table 1), but another prominent *m/z* in the mass spectrum may also be used for quantitation provided it is used consistently. If interferences are noted at the primary *m/z*, use an alternate *m/z*. Calibration range, chromatographic performance, and extent of any carryover will depend on the introduction technique, GC column and conditions, and the tolerance of the sample introduction system and GC/MS to solvent, water, and other introduced sample matrix components.

NOTE: LLOQs should be established at concentrations where both quantitative and qualitative verifications can be consistently and reliably met (see Secs. 9.9 and

11.6). Target analyte peaks in the calibration standard at the LLOQ should be visually inspected to ensure that each peak signal is distinguishable from background and to verify qualitative analyte identification.

11.3.3 Additional considerations for SIM and SRM analysis

SIM and SRM may be useful for applications requiring quantitation limits below the normal range of electron impact quadrupole mass spectrometry, and both are allowable options for this method. Using the primary *m/z* (or product ion for SRM detectors) for quantitation and at least one secondary *m/z (or product ion)* for confirmation, set up the collection groups based on their chromatographic RTs. The selected *m/z* (or product ion) values should include any mass defect noted in the target analyte mass spectra acquired on the instrument, usually less than 0.2 amu. The dwell time for each ion may be automatically calculated by the instrument software or may be calculated based on the peak widths of the analytes of interest, the number of spectra needed to be acquired across each peak, and the number of concurrent ions that need to be acquired in each segment. When fewer *m/z* values are monitored in each segment, the acquisition time for each *m/z* can be increased, thereby increasing the sensitivity of the system. The total cycle time for the MS should be short enough that at least five, but preferably ten or more, spectra are acquired per chromatographic peak.

When compounds are analyzed in SIM or SRM mode, the following best practices are recommended:

- Monitor at least two ions for each target analyte, and use the mid-point of the calibration curve to establish proper ion ratios for each compound. The ratios of primary and secondary ions are the only qualitative tools available in SIM and SRM runs (other than RT), which increases their importance in proper identification. When interferences are expected or observed in a given matrix, acquiring multiple secondary ions may aid in qualitative identification.

- Verify that all monitored ions are correctly integrated in order to achieve proper ion ratios. Update the primary/secondary ion ratios and reference mass spectra after each ICAL using a mid-range ICAL standard.

11.3.4 Tabulate the response of the characteristic ions (see Table 1 for suggested ions) against the concentration for each target analyte and each IS. Calculate RFs for each target analyte relative to one of the ISs as follows:

$$RF = \frac{A_s \times C_{is}}{A_{is} \times C_s}$$

where:

A_s = Peak response of the analyte or surrogate
A_{is} = Peak response of the IS
C_s = Concentration of the analyte or surrogate
C_{is}= Concentration of the IS

11.3.4.1 Calculate the mean RF and the relative standard deviation (RSD) of the RFs for each target analyte using the following equations.

$$\text{mean RF} = \overline{RF} = \frac{\sum_{i=1}^{n} RF_i}{n} \qquad RSD = \frac{SD}{\overline{RF}} \times 100 \qquad SD = \sqrt{\frac{\sum_{i=1}^{n}(RF_i - \overline{RF})^2}{n-1}}$$

where:

RF_i = RF for each of the calibration standards
$\overline{RF}$ = mean RF for each compound from the ICAL
n = Number of calibration standards, e.g., 5
SD = Standard deviation

11.3.4.2 The RSD should be ≤ 20% for each target analyte (see Sec. 11.3.5). Table 4 contains minimum RFs that may be used as guidance in determining whether the system is behaving properly and as a check to see if calibration standards are prepared correctly. Because the minimum RFs in Table 4 were determined using specific ions and instrument conditions that may vary, it is neither expected nor required that all analytes meet these minimum RFs. The information in this table is provided as guidance only. The laboratory should establish procedures in its determinative SOP (e.g., laboratory established minimum RFs, signal to noise (S/N) checks, etc.) to ensure that the instrument is working properly and that calibration standards were correctly prepared.

NOTE: For a target analyte whose RF <0.01 (response of peak is <1/100 the response of the IS), it is recommended to increase its concentration in relation to other analytes to make the response more comparable.

11.3.5 Linearity of target analytes – If the RSD of any target analyte is ≤ 20%, then the RF is assumed to be constant over the calibration range, and the average RF may be used for quantitation (Sec. 11.7.2).

11.3.5.1 If the RSD of any target analyte RF is >20%, refer to Sec. 11.5 of Method 8000 for additional calibration options (e.g., narrowing the calibration range, changing calibration model, etc.), and apply one or more of these options in order to meet the ICAL acceptance criteria. Alternatively, the affected target analytes may be reported with an appropriate data qualifier, or the instrument may be recalibrated.

NOTE: When the RSD for the RF calibration model is >20%, plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

NOTE: Forcing the calibration model through the origin (for analytes that are consistently detected in the laboratory reagent blanks) allows for a better estimate of the background level of blank contaminants. An accurate estimate of background contamination is necessary to set method reporting limits for method analytes when blank levels are problematic.

11.3.5.2 If more than 10% of the compounds included with the ICAL (or more than 10% of those that will be reported) exceed the 20% RSD limit and do

not meet the minimum correlation criteria (r^2 ≥0.99 or relative standard error (RSE) ≤ 20%) for alternate curve fits, then the chromatographic system is considered too reactive for analysis to begin. Correct the source of the problem; then repeat the calibration procedure beginning with Sec. 11.3. If compounds fail to meet these criteria, the associated concentrations may still be determined but they must be reported as estimated. In order to report non-detects, it must be demonstrated that there is sufficient accuracy to detect the failed compounds at the applicable LLOQ (see Secs. 11.3.5.4 for refitting standards and 11.4.3.2 for CCV). Refer to Method 8000 for further discussion of RSE. Example RSE calculations can be found in Reference 16.

11.3.5.3 Due to the large number of compounds that may be analyzed by this method, it is likely that some compounds will not meet the acceptance criteria described above. For these occasions, it is acknowledged that those compounds that do not meet the criteria may not be critical to the specific project and therefore data generated may be used as qualified data or estimated values for screening purposes. The analyst should strive to place more emphasis on meeting the calibration criteria for those compounds that are critical to the project. The target analytes that do not meet the ICAL criteria should still be identified to the data user and the resulting data qualified appropriately, but it is not necessary to meet criteria for compounds that will not be reported.

NOTE: It is considered inappropriate, once the calibration models have been finalized, to select an alternate fit solely to pass the recommended QC criteria for samples and associated QC on a case-by-case basis.

11.3.5.4 Calibration, especially when using linear regression models, has the potential for a significant bias at the lower portion of the calibration curve. All calibration points, especially those equivalent to the LLOQ, should be recalculated (not reanalyzed) using the final calibration curve in which this standard is used (i.e., re-fitting the response from the calibration standard back into the curve). See Method 8000 for additional details. The recalculated concentration of the calibration standard corresponding to the LLOQ, especially where linear regression fits are used, should be within ±50% of the standard's true concentration if it is the lowest point, and within ±30% for all others (i.e. above the low standard). No refit criteria need be passed for calibration levels below the LLOQ. Alternate criteria may be applied depending on the needs of the project. However, those criteria should be clearly defined in a laboratory SOP or a project-specific QAPP. Analytes which do not meet the re-fitting criteria should be evaluated for corrective action. If a failure occurs in the low point and it is equivalent to the LLOQ, the analyte should be reported as estimated near that concentration or the LLOQ should be reestablished at a higher concentration (See Method 8000 Sec. 11.5.4 for calculations).

11.3.6 ICV – Prior to analyzing samples, verify the ICAL using a standard obtained from a second source to the calibration standard, if possible, such as a second manufacturer or a manufacturer's batch prepared independently from the batch used for calibration, if readily available. This standard should be prepared in the same clean control matrix as that used for ICAL standards. Suggested acceptance criteria for the analyte concentrations in this standard are 70 - 130% of the expected analyte concentration(s). Alternative criteria may be appropriate based on project-specific DQOs. Quantitative sample analyses should not proceed for those analytes that do not meet the ICAL verification criteria. However, analyses may continue for those analytes that do not meet the criteria with an understanding that these results could be used for

screening purposes and would be considered estimated values.

11.4 CCV – A CCV standard must be analyzed at the beginning of each twelve-hour analytical period prior to any sample analysis.

NOTE: Tune checks (Sec. 11.3.1) are only required prior to ICAL.

11.4.1 The ICAL function (Sec. 11.3) for each compound of interest must be verified once every twelve hours prior to sample analysis, using the same introduction technique and conditions as used for analysis of ICAL standards and samples. This is accomplished by analyzing a CCV standard (containing all the compounds that will be reported) prepared from the same stock solutions or source materials used for ICAL standards and at a concentration near the midpoint of the ICAL range. The results must be compared against the most recent calibration curve and should meet the CCV acceptance criteria provided in Secs. 11.4.3-11.4.5.

NOTE: This QC check may be omitted if samples are analyzed within twelve hours of ICAL, and injection of the last ICAL standard may be used as the starting time reference for evaluation.

11.4.2 A blank must also be analyzed after the CCV standard and prior to any samples in order to demonstrate that the total system (introduction device, transfer lines and GC/MS system) is free from contaminants. Analytes of interest for the project that did not meet the criteria should be identified to the data user and results qualified appropriately. If the blank indicates contamination, then it may be appropriate to analyze additional blanks to reduce any system contamination due to carryover from standards or samples. See Sec. 9.5 for MB performance criteria. See Method 8000 for information regarding MB performance criteria.

11.4.3 CCV standard criteria

11.4.3.1 The calculated concentration or amount of each analyte of interest in the CCV standard should fall within ±20% of the expected value.

NOTE: For the RF calibration model, % difference (%D) between the calculated RF of an analyte in the calibration verification standard and the RF_{avg} of that analyte from the ICAL is the same value as % drift for calculated vs. expected concentration. Refer to Method 8000 for guidance on calculating %D and % drift.

11.4.3.2 If the %D or % drift for a compound is ≤20%, then the ICAL for that compound is assumed to be valid. Due to the large numbers of compounds that may be analyzed by this method, it is expected that some compounds will fail to meet the criterion. The analyst should strive to place more emphasis on meeting the CCV criteria for those compounds that are critical to the project. If the criterion is not met (i.e., greater than ±20%D or drift), for more than 20% of the compounds included in the ICAL (or more than 20% of those that will be reported), then corrective action must be taken prior to the analysis of samples. Target analytes that do not meet the CCV criteria and are reported in the associated samples must be qualified to indicate the reported concentrations are potentially estimated or biased values. In cases where compounds fail low, they may be reported as non-detects if it can be demonstrated that there was adequate sensitivity to detect the compound at the LLOQ or project specific level of interest (e.g., by calibrating below the established LLOQ to confirm the non-

detect, or by analyzing a standard near that level to confirm the analyte could be qualitatively identified if it were present [See Sec. 11.7 of Method 8000]). Alternatively, the non-detect could be qualified or the LLOQ raised to a higher level. In cases where compounds fail high in the CCV and are not found in the associated field samples, they may be reported without qualification.

NOTE: If significant losses of target analytes/ISs occur (<50% recovery) or if significant degradation of the chromatography occurs, system maintenance must be performed, or the analyst must demonstrate there is adequate sensitivity at the LLOQ.

11.4.3.3 Problems similar to those listed under ICAL could affect the ability to pass the CCV criteria. If the problem cannot be corrected by other measures, a new ICAL must be generated. The calibration verification criteria must be met before sample analysis begins.

11.4.4 IS RT – If the absolute RT for any IS in the CCVs changes by more than 30 seconds from that in the mid-point standard level of the most recent ICAL sequence, then the chromatographic system must be inspected for malfunctions and corrections must be made, as required. When corrections are made, reanalysis of samples analyzed while the system was malfunctioning is required. RT changes will depend on the type of chromatographic system used.

11.4.5 IS responses – In order to demonstrate continued stability of the measurement system after ICAL, IS responses in the CCVs must be evaluated by comparing them to the responses of the same ISs in the ICAL standard(s). If the response of an IS changes by more than a factor of 2 (50 - 200%) relative to the response of that IS in the mid-point ICAL standard or the average of responses in the suite of ICAL standards (as defined in the laboratory's SOP), then corrective actions should be taken. These corrective actions may include but are not limited to replacing and/or reanalyzing the CCV standard, or retuning the MS and re-calibrating the instrument. When IS responses do not meet these criteria, system sensitivity may have been compromised, and sample reanalysis is recommended, especially if any action limits for the project are near the LLOQ.

11.5 GC/MS analysis of samples

11.5.1 It is highly recommended that samples be screened to minimize contamination of the GC/MS system or sample introduction device from unexpectedly high concentrations of organic compounds. Some of the screening options available utilizing SW-846 methods are:

-Screening solid samples for VOCs (Method 3815), automated headspace,
-GC/flame ionization detector (FID) (Methods 5021/8015), automated headspace,
-GC/photo ionization detector (PID)/electrolytic conductivity detector (ELCD) (Methods 5021/8021), or,
-Waste dilution - GC/PID/ELCD (Methods 3585/8021) using the same type of capillary column.

When used only for screening purposes, the QC requirements in the methods above may be reduced as appropriate. Sample screening is particularly important when Method 8260 is used to achieve low quantitation levels.

11.5.2 Add appropriate volumes of the surrogates spiking solution and the IS

spiking solution to each field sample and all associated QC samples either manually or by an autosampler to achieve the desired concentrations. The surrogates and ISs may be mixed and added as a single spiking solution.

11.5.3 Add an aliquot of the target compounds spiking solution (Sec. 7.12) to any sample aliquot(s) chosen for matrix spiking. Follow the same procedure in preparing the LCS, adding the spike to the same clean control material used for calibration standards preparation (e.g., reagent water, Ottawa sand, etc.). See Secs. 7.12, 9.6.2 and Method 8000 for more guidance on the selection and preparation of the matrix spike and the LCS. The LCS for solid matrices may be prepared in clean sand or organic-free reagent water. However, the use of sand is not required.

11.5.4 Introduce field samples and associated QC samples to the GC/MS under the same conditions used for analysis of ICAL standards. When screening results indicate high levels of target analytes and/or interferences, or if analyte concentrations are measured above the calibration range, prepare and analyze an appropriate dilution of the sample(s), or choose a preparation method that is more amenable to making dilutions (e.g., methanol extraction of solids instead of direct aqueous partitioning). Dilutions should be targeted so the response of the major constituents (previously saturated peaks) falls near the middle of the calibration range.

11.5.5 When the concentration of a compound in the sample is high enough to result in significant carryover to subsequent samples (Sec. 9.5), this analysis should be followed by at least one MB or instrument blank to demonstrate lack of carryover to the proceeding field sample. If analysis of one or more blanks is not sufficient to return the system to acceptable operating conditions, more extensive decontamination procedures may be required, and subsequent recalibration may be necessary. Alternatively, when analysis of a blank is not possible prior to the next sample, such as when an unattended autosampler is employed, the analyst should review the results for at least the next sample after the high-concentration sample. If analytes in the high-concentration sample are not present in the subsequent field sample, then the lack of carryover has been demonstrated.

11.5.6 IS responses and RTs should be monitored in all field samples and associated QC samples in order to provide sample-specific QA of proper analyte introduction to the GC/MS system and to anticipate the need for system inspection and/or maintenance. If the response of the primary *m/z* for any of the ISs in the field samples or associated QC samples varies by more than a factor of two (50% - 200%) from that of the same IS in the mid-point ICAL standard, average of ICAL standards, or most recently analyzed CCV standard (as defined in the laboratory's SOP), corrective action should be taken. Any affected field samples and associated QC samples should be re-analyzed, or the associated data should be qualified.

11.6 Analyte identification

11.6.1 Qualitative identification of each compound determined by this method is based on RT and on comparison of the sample mass spectrum, after background correction, with a reference mass spectrum. Compounds are identified as present when the following criteria are met.

11.6.1.1 The intensities of the characteristic ions of a compound maximize in the same mass spectra or in adjacent mass spectra.

11.6.1.2 The RT is within ±10 seconds of the RT for this analyte in the

midpoint ICAL standard or CCV standard analyzed at the beginning of the 12-hour period (delta RT 0.17 minute), or within ±10 seconds relative to the shift of the associated IS (delta RT of the IS ±10 seconds). Chromatograms should be carefully inspected to minimize the occurrence of both false positive and false negative results. If the RT for the IS has shifted, the sample should be inspected for similar shifts for the associated target analytes. If RT drift is significant, relative retention time (RRT) may be useful as an alternative to delta retention times. See Section 11.4 of Method 8000 for additional information.

NOTE: Some analytes may have RT shifting that is much greater than the associated IS (greater than ±10 seconds relative to the IS shift) and is still the target analyte. In those cases, it may be more useful to compare the delta RT with compounds that have similar chemistries to help identify the target. Also, dilutions or spiked samples are recommended to help determine the effects of matrix on the elution of the target and assist in target identification.

11.6.1.3 The relative intensities of the qualifier ion(s) (*i.e.*, secondary characteristic ions or alternate MS/MS transitions) should agree within 30% of the relative intensities of these ions in the reference spectrum. For example, for a qualifier ion with a response of 50% of the quantitation ion in the reference spectrum, the corresponding qualifier ion ratio in a sample spectrum can range between 20% and 80%. The reference mass spectrum used for this comparison should be generated by the laboratory using the conditions of this method (typically a mid-level calibration standard). Qualitative identification of sample mass spectra not acquired in limited ion acquisition modes (i.e., SIM or SRM) may also be supported by comparison to a reference library as described in Sec. 11.6.2.

11.6.1.4 Unresolved structural isomers with similar mass spectra are identified as isomeric pairs. Isomers are considered resolved if the peaks are at least 50% resolved (i.e., the height of the valley between two isomer peaks is ≤ 50% of the average of the two peak heights, or 1−[valley height]/[average peak height] is ≥50%). The resolution should be verified on the mid-point concentration of the ICAL as well as the laboratory-designated CCV level if closely eluting isomers are to be reported. It is important to check the separation of structural isomers in the ICV and the daily CCV check standards to verify if the instrument performance is adequate regarding separation of compounds of interest which are structural isomers.

11.6.1.5 Identification is hampered when sample components are not resolved chromatographically and produce mass spectra containing ions contributed by more than one analyte. When gas chromatographic peaks obviously represent more than one sample component (i.e., a broadened peak with shoulder(s) or a valley between two or more maxima), appropriate selection of analyte spectra and background spectra is important.

11.6.1.6 Examination of EICPs of appropriate ions can aid in the selection of spectra, and in qualitative identification of compounds. When analytes co-elute (i.e., only one chromatographic peak is apparent), the identification criteria may be met, but each analyte spectrum will contain extraneous ions contributed by the co-eluting compound.

11.6.2 For samples containing components not associated with the calibration standards, a library search may be made for the purpose of tentative identification. The necessity to perform this type of identification will be determined by the purpose of the analyses being conducted (for example, the RCRA permit or waste delisting requirements may require the reporting of non-target analytes). Data system library search routines should not use normalization routines that would misrepresent the library or unknown spectra when compared to each other. Only after visual comparison of sample spectra with library search results may the analyst assign a tentative identification. Use the following guidelines for making tentative identifications:

(1) Major ions in the library reference spectrum (ions greater than 10% of the most abundant ion) are present in the sample spectrum at similar relative intensities.

(2) The molecular ion in the library reference spectrum is present in the sample spectrum. If the molecular ion is not present, carefully review library matches in order to avoid misidentification.

(3) Major ions present in the sample spectrum but not in the reference spectrum are reviewed to determine whether they may be contributed by co-eluting compounds.

(4) Ions present in the reference spectrum but not in the sample mass spectra are reviewed for unintended subtraction. Data system library reduction programs can sometimes create these discrepancies.

(5) Mass spectral library search algorithms typically assign a match factor to the peak identity based on comparison of an unknown mass spectrum to library spectra. For spectra meeting the above conditions, match factors greater than 0.8 (80%) may be considered confirming evidence. Where a known limitation in data collection is identified (e.g., the presence of an incompletely resolved spectral interference), a lower match factor may be considered confirmatory. For multiple library spectra with similar match factors (e.g., for hydrocarbons with low abundance molecular ions, or structural isomers), the tentative identification assigned to the unknown may be better represented as a more generic structure (e.g., unknown hydrocarbon, C4 benzene structural isomer). See Reference 15 for more information.

11.7 Quantitation

11.7.1 Once a compound has been identified, the quantitation of that compound will be based on the integrated abundance from the EICP of the primary characteristic ion. The IS used should be the one nearest the RT of that of a given analyte.

11.7.1.1 Where the integration produced by the software is acceptable, it is recommended to use it, because the software should produce more consistent integrations. Manual integrations are necessary when the software does not properly integrate peaks, such as when the baseline selection is improper; the correct peak is missed; a co-elution is integrated; the peak is partially integrated; etc. The analyst is responsible for ensuring that the integration is correct whether performed by the software or done manually.

11.7.1.2 Manual integrations should not be substituted for proper maintenance of the instrument or setup of the method (e.g., RT updates, integration parameter files, etc.). The analyst should seek to minimize manual integration by properly maintaining the instrument, updating RTs, and configuring peak integration parameters.

11.7.2 If the RSD is 20% or less, then the RF calibration model is acceptable for the ICAL (Sec. 11.3.4). See Method 8000 for the equations describing IS calibration and either linear or non-linear calibrations.

11.7.3 Where applicable, the concentrations of any non-target analytes identified in the sample (Sec. 11.6.2) may be estimated using the RF calibration model formula, with the following modifications: The responses A_x and A_{is} as defined in Sec.11.3.4 should be from the total ion chromatograms, and the RF for the non-target analyte should be assumed to be 1. The resulting concentration should be clearly identified as an estimate. Use the nearest IS free of interferences.

11.7.4 Structural isomers that produce very similar mass spectra may be quantitated as individual isomers if they are sufficiently resolved. See Sec. 11.6.1.4.

11.7.5 Quantitation of multicomponent parameters such as gasoline-range organics (GROs) and total petroleum hydrocarbons (TPH) using the Method 8260-recommended IS quantitation technique is beyond the scope of this method. Typically, analyses for these parameters are performed using a GC/FID or GC with a MS detector capability as is described in Method 8015. However, it is acceptable to use the total ion chromatogram that is generated from this method with external standard calibration to quantitate such parameters. External standard calibration is recommended for these applications in order to reduce the need to subtract area contributed by multiple non-target peaks (such as the ISs) in the TPH chromatogram. See Sec. 11.4.2 in Method 8000 and Method 8015 for additional guidance.

12.0 DATA ANALYSIS AND CALCULATIONS

See Sec. 11.7 for information on data analysis and calculations.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.

13.2 This method has been tested using purge and trap (Method 5030) in a single laboratory using spiked water. Using a wide-bore capillary column, water was spiked at concentrations between 0.5 and 10 µg/L. Single laboratory accuracy and precision data for the method analytes are available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry.

13.3 Direct injection (Method 3585) has been used for the analysis of waste motor oil samples using a wide-bore column. Single laboratory precision and accuracy data are

available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry for toxicity characteristic leaching procedure (TCLP) volatiles in oil. The performance data were developed by analyzing seven replicates each of new and used oil. The oils were spiked at the TCLP regulatory concentrations for most analytes, with the exceptions of the alcohols, ketones, ethyl acetate and chlorobenzene which are spiked at 5 ppm (well below the regulatory concentrations). Prior to spiking, the new oil (i.e., a Society of Automotive Engineers (SAE) 30-weight motor oil) was heated at 80 °C overnight to remove volatiles. The used oil (i.e., a mixture of used oil drained from passenger automobiles) was not heated and was contaminated with 20 - 300 ppm of benzene, toluene, ethylbenzene and xylene (BTEX) compounds and isobutanol. These contaminants contributed to high recoveries of the BTEX compounds in the used oil. Therefore, the data from the deuterated analogs of these analytes represent more typical recovery values.

13.4 Single laboratory accuracy and precision data were obtained for the Method 5035 analytes in three soil matrices: sand, a soil collected 10 feet below the surface of a hazardous waste landfill, and a surface garden soil. Sample preparation was by Method 5035. Each sample was fortified with the analytes at a concentration of 20 µg/kg. These data are available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry. All data were calculated using fluorobenzene, added to the soil sample prior to methanol extraction, as the IS. Some of the results were greater than 100% recovery, likely due to variance in IS response.

13.4.1 In general, the recoveries of the analytes from the sand matrix are the highest, the hazardous waste landfill soil results are somewhat less, and the surface garden soil recoveries are the lowest. This is due to the greater adsorptive capacity of the garden soil. This illustrates the necessity of analyzing matrix spike samples to assess the degree of matrix effects.

13.4.2 The recoveries of some of the gases, or very volatile compounds, such as vinyl chloride, trichlorofluoromethane, and 1,1-dichloroethene, were somewhat greater than 100%, likely due to the difficulty encountered in fortifying the soil with these compounds, allowing an equilibration period, then extracting them with a high degree of precision. The garden soil results (available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry) also include high recoveries for some aromatic compounds, including toluene, xylenes, and trimethylbenzenes. This is likely due to high levels of contamination of the soil prior to sample collection.

13.5 Performance data for non-purgeable volatiles using azeotropic distillation (Method 5031) are included in Reference 9.

13.6 Performance data for volatiles prepared using vacuum distillation (Method 5032) in soil, water, oil, and fish tissue matrices are included in Reference 11.

13.7 Single laboratory accuracy and precision data were obtained for the Method 5021 analytes in a garden soil matrix. Replicate samples were fortified with the analytes at a concentration of 20 µg/kg. These data are available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry. The recommended ISs were selected because they generated the best accuracy and precision data for the analytes in both types of soil.

13.7.1 Example LLOQs using Method 5021 are available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry and were calculated from results of seven

replicate analyses of the sand matrix. Sand was chosen because it demonstrated the least degree of matrix effect of the soils studied. These LLOQs were calculated utilizing the procedure described in Chapter One and are intended to be a general indication of the capabilities of the method.

13.8 The LLOQ for samples taken by Method 0040 and analyzed by Method 8260 is estimated to be in the range of 0.03 to 0.9 parts-per-million (ppm). Data can be found at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry. Matrix effects may cause the individual compound quantitation limits to be higher.

13.9 The recommended ISs with corresponding analytes assigned for quantitation that are appropriate for Method 5041 are available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult: http://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available at: http://www.labsafety.org/FreeDocs/WasteMgmt.pdf.

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

Table 1

CHARACTERISTIC MASSES (*m/z*) FOR PURGEABLE ORGANIC COMPOUNDS

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Acetone	58	43
Acetonitrile	41	40, 39
Acrolein (Propenal)	56	55, 58
Acrylonitrile	53	52, 51
Allyl alcohol	57	58, 39
Allyl chloride	76	41, 39, 78
Benzene	78	-
Benzyl chloride	91	126, 65, 128
Bromoacetone	136	43, 138, 93, 95
Bromobenzene	156	77, 158
Bromochloromethane	128	49, 130
Bromodichloromethane	83	85, 127
Bromoform	173	175, 254
Bromomethane	94	96
iso-Butanol	74	43
n-Butanol (1-Butanol, *n*-Butyl alcohol)	56	41
2-Butanone	72	43
n-Butylbenzene	91	92, 134
sec-Butylbenzene	105	134
tert-Butylbenzene	119	91, 134
Carbon disulfide	76	78
Carbon tetrachloride	117	119
Chloral hydrate	82	44, 84, 86, 111
Chloroacetonitrile	48	75
Chlorobenzene	112	77, 114
1-Chlorobutane	56	49
Chlorodibromomethane	129	208, 206
Chloroethane	64 (49*)	66 (51*)
2-Chloroethanol	49	44, 43, 51, 80
Bis(2-chloroethyl) sulfide	109	111, 158, 160
2-Chloroethyl vinyl ether	63	65, 106
Chloroform	83	85
Chloromethane	50 (49*)	52 (51*)
Chloroprene	53	88, 90, 51
3-Chloropropionitrile	54	49, 89, 91
2-Chlorotoluene	91	126
4-Chlorotoluene	91	126
1,2-Dibromo-3-chloropropane (DBCP)	75	155, 157
Dibromochloromethane	129	127
1,2-Dibromoethane (EDB, Ethylene dibromide)	107	109, 188

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Dibromomethane	93	95, 174
1,2-Dichlorobenzene	146	111, 148
1,3-Dichlorobenzene	146	111, 148
1,4-Dichlorobenzene	146	111, 148
cis-1,4-Dichloro-2-butene	75	53, 77, 124, 89
trans-1,4-Dichloro-2-butene	53	88, 75
Dichlorodifluoromethane	85	87
1,1-Dichloroethane	63	65, 83
1,2-Dichloroethane	62	98
1,1-Dichloroethene (Vinylidene chloride)	96	61, 63
cis-1,2-Dichloroethene	96	61, 98
trans-1,2-Dichloroethene	96	61, 98
1,2-Dichloropropane	63	112
1,3-Dichloropropane	76	78
2,2-Dichloropropane	77	97
1,3-Dichloro-2-propanol	79	43, 81, 49
1,1-Dichloropropene	75	110, 77
cis-1,3-Dichloropropene	75	77, 39
trans-1,3-Dichloropropene	75	77, 39
1,2,3,4-Diepoxybutane	55	57, 56
Diethyl ether	74	45, 59
1,4-Dioxane	88	58, 43, 57
Epichlorohydrin	57	49, 62, 51
Ethanol	31	45, 27, 46
Ethyl acetate	88	43, 45, 61
Ethyl benzene	91	106
Ethyl methacrylate	69	41, 99, 86, 114
Ethylene oxide	44	43, 42
Hexachlorobutadiene	225	223, 227
Hexachloroethane	201	166, 199, 203
2-Hexanone	43	58, 57, 100
2-Hydroxypropionitrile	44	43, 42, 53
Iodomethane (Methyl iodide)	142	127, 141
Isobutyl alcohol (2-Methyl-1-propanol)	43	41, 42, 74
Isopropylbenzene	105	120
p-Isopropyltoluene	119	134, 91
Malononitrile	66	39, 65, 38
Methacrylonitrile	41	67, 39, 52, 66
Methyl acrylate	55	85
Methyl-*t*-butyl ether	73	57
Methyl iodide (Iodomethane)	142	127, 141
Methyl methacrylate	69	41, 100, 39
4-Methyl-2-pentanone	100	43, 58, 85
Methylene chloride	84	86, 49

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Naphthalene	128	-
Nitrobenzene (NB)	123	51, 77
2-Nitropropane	46	-
Pentachlororethane	167	130, 132, 165, 169
2-Picoline (2-Methylpyridine)	93	66, 92, 78
Propargyl alcohol	55	39, 38, 53
β-Propiolactone	42	43, 44
Propionitrile (Ethyl cyanide)	54	52, 55, 40
n-Propylamine	59	41, 39
n-Propylbenzene	91	120
Pyridine	79	52
Styrene	104	78
1,1,1,2-Tetrachloroethane	131	133, 119
1,1,2,2-Tetrachloroethane	83	131, 85
Tetrachloroethene	164	129, 131, 166
Toluene	92	91
1,2,3-Trichlorobenzene	180	182, 145
1,2,4-Trichlorobenzene	180	182, 145
1,1,1-Trichloroethane	97	99, 61
1,1,2-Trichloroethane	83	97, 85
Trichlororethene	95	97, 130, 132
Trichlorofluoromethane	101	103
1,2,3-Trichloropropane	75	77
1,2,4-Trimethylbenzene	105	120
1,3,5-Trimethylbenzene	105	120
Vinyl acetate	43	86
Vinyl chloride	62	64
o-Xylene	106	91
m-Xylene	106	91
p-Xylene	106	91

Table 1A

Internal Standards/Surrogates

Compound	Primary Characteristic Ion	Secondary Characteristic Ion(s)
Benzene-d_5	84	83
Bromobenzene-d_5	82	162
Bromochloromethane-d_2	51	131
4-Bromofluorobenzene	95	174, 176
Chlorobenzene-d_5	117	-
Chloroform-d_1	84	-
Dibromofluoromethane	113	-
1,2-Dichlorobenzene-d_4	152	115, 150
1,4-Dichlorobenzene-d_4	152	115, 150
Dichloroethane-d_4	102	-
1,4-Difluorobenzene	114	-
Fluorobenzene	96	77
Pentafluorobenzene	168	-
Toluene-d_8	98	-
1,1,2-Trichloroethane-d_3	100	-

*Characteristic ion for an ion trap MS (to be used when ion-molecule reactions are observed).

Table 2

2012 STATISTICAL STUDY OF LABORATORY CONTROL SAMPLE (LCS) LIMITS FROM DOD LCS DATA

Relative standard deviation of recoveries by analyte for compounds where the number of replicates, N was greater than 20 (average for all analytes: Recovery = 97%, 12% RSD). See Ref. #13 for details on EPA statistical study of DOD LCS data.

Analyte Name	CAS #	Water RSD	Solid RSD
Acetaldehyde	75-07-0	66%	52%
Acetone	67-64-1	20%	21%
Acetonitrile	75-05-8	16%	15%
Acrolein [Propenal]	107-02-8	20%	18%
Acrylonitrile	107-13-1	12%	11%
Allyl chloride	107-05-1	11%	11%
tert-Amyl ethyl ether	919-94-8	10%	10%
tertiary-Amyl methyl ether (tame)	994-05-8	6%	10%
Benzene	71-43-2	7%	7%
Benzyl chloride	100-44-7	18%	10%
Bis(2-chloro-1-methylethyl) ether	108-60-1	12%	NA
Bromobenzene	108-86-1	7%	7%
Bromochloromethane	74-97-5	7%	8%
Bromodichloromethane	75-27-4	8%	8%
4-Bromofluorobenzene	460-00-4	5%	7%
Bromoform	75-25-2	11%	11%
Bromomethane	74-83-9	15%	15%
1,3-Butadiene	106-99-0	19%	10%
2-Butanone [MEK]	78-93-3	15%	16%
n-Butyl acetate	123-86-4	10%	12%
Butyl acrylate	141-32-2	72%	NA
n-Butyl alcohol	71-36-3	13%	14%
sec-Butyl alcohol	78-92-2	NA	17%
tert-Butyl alcohol	75-65-0	10%	11%
tert-Butyl formate	762-75-4	11%	NA
sec-Butylbenzene	135-98-8	8%	9%
tert-Butylbenzene	98-06-6	8%	9%
Carbon disulfide	75-15-0	12%	12%
Carbon tetrachloride	56-23-5	10%	11%
Chlorobenzene	108-90-7	6%	7%
2-Chloro-1,3-butadiene	126-99-8	12%	11%
Chlorobutane	109-69-3	NA	8%
Chlorodibromomethane	124-48-1	9%	9%
Chlorodifluoromethane	75-45-6	18%	19%
Chloroethane	75-00-3	13%	13%
2-Chloroethyl vinyl ether	110-75-8	16%	18%

Analyte Name	CAS #	Water RSD	Solid RSD
Chloroform	67-66-3	7%	8%
1-Chlorohexane	544-10-5	8%	10%
Chloromethane	74-87-3	16%	15%
1-Chloropropane	540-54-5	10%	8%
2-Chloropropane	75-29-6	12%	9%
2-Chlorotoluene	95-49-8	7%	8%
4-Chlorotoluene	106-43-4	7%	9%
2-Chloro-1,1,1-trifluoroethane	75-88-7	8%	7%
Chlorotrifluoroethene	79-38-9	22%	24%
Cyclohexane	110-82-7	10%	11%
Cyclohexanone	108-94-1	42%	22%
1,2-Dibromo-3-chloropropane	96-12-8	12%	12%
1,2-Dibromoethane (EDB, Ethylene dibromide)	106-93-4	7%	7%
Dibromofluoromethane	1868-53-7	7%	7%
Dibromomethane	74-95-3	7%	8%
1,2-Dichlorobenzene	95-50-1	7%	7%
1,3-Dichlorobenzene	541-73-1	7%	8%
1,4-Dichlorobenzene	106-46-7	7%	8%
cis-1,4-Dichloro-2-butene	1476-11-5	15%	12%
trans-1,4-Dichloro-2-butene	110-57-6	18%	13%
Dichlorodifluoromethane [Freon-12]	75-71-8	22%	23%
1,1-Dichloroethane	75-34-3	8%	8%
1,2-Dichloroethane	107-06-2	9%	9%
1,1-Dichloroethene (Vinylidene chloride)	75-35-4	10%	10%
1,2-Dichloroethene	540-59-0	7%	7%
cis-1,2-Dichloroethene	156-59-2	8%	8%
trans-1,2-Dichloroethene	156-60-5	8%	9%
Dichlorofluoromethane	75-43-4	10%	18%
1,2-Dichloropropane	78-87-5	7%	8%
1,3-Dichloropropane	142-28-9	7%	7%
2,2-Dichloropropane	594-20-7	13%	11%
1,1-Dichloropropene	563-58-6	8%	8%
1,3-Dichloropropene	542-75-6	8%	8%
cis-1,3-Dichloropropene	10061-01-5	8%	9%
trans-1,3-Dichloropropene	10061-02-6	9%	10%
1,2-Dichloro-1,1,2,2-tetrafluoroethane	76-14-2	16%	20%
1,2-Dichlorotrifluoroethane [Freon 123a]	354-23-4	11%	12%
Diethyl ether	60-29-7	10%	10%
Diethylbenzene (total)	25340-17-4	6%	6%
1,2-Diethylbenzene	135-01-3	6%	5%
1,3-Diethylbenzene	141-93-5	6%	6%
1,4-Diethylbenzene	105-05-5	6%	6%
Diisopropyl ether	108-20-3	11%	10%

Analyte Name	CAS #	Water RSD	Solid RSD
Dimethyl ether	115-10-6	11%	NA
3,3-Dimethyl-1-butanol	624-95-3	15%	14%
Dimethyldisulfide	624-92-0	15%	9%
1,4-Dioxane	123-91-1	14%	14%
Epichlorohydrin	106-89-8	13%	14%
Ethanol	64-17-5	17%	19%
Ethyl acetate	141-78-6	14%	15%
Ethyl acrylate	140-88-5	38%	49%
Ethyl methacrylate	97-63-2	9%	10%
Ethyl tert-butyl ether	637-92-3	10%	9%
Ethylbenzene	100-41-4	7%	8%
2-Ethyl-1-hexanol	104-76-7	21%	25%
4-Ethyltoluene	622-96-8	14%	13%
Fluorobenzene	462-06-6	6%	6%
Furan	110-00-9	16%	NA
Heptane	142-82-5	16%	16%
Hexachlorobutadiene	87-68-3	11%	13%
Hexachloroethane	67-72-1	10%	10%
Hexane	110-54-3	17%	17%
2-Hexanone	591-78-6	14%	16%
Iodomethane (Methyl iodide)	74-88-4	10%	10%
Isoamyl alcohol	123-51-3	14%	14%
Isobutyl alcohol	78-83-1	12%	13%
Isoprene	78-79-5	9%	18%
Isopropyl acetate [Acetic acid]	108-21-4	12%	13%
Isopropylbenzene	98-82-8	10%	11%
p-Isopropyltoluene [p-Cymene]	99-87-6	8%	9%
Methacrylonitrile	126-98-7	12%	11%
Methyl acetate	79-20-9	14%	15%
Methyl acrylate	96-33-3	12%	11%
Methyl methacrylate	80-62-6	10%	12%
Methyl sulfide	75-18-3	13%	12%
Methyl tert-butyl ether [MTBE]	1634-04-4	9%	9%
Methylcyclohexane	108-87-2	10%	11%
Methylene chloride	75-09-2	8%	10%
2-Methylnaphthalene	91-57-6	26%	27%
4-Methyl-2-pentanol	108-11-2	15%	NA
4-Methyl-2-pentanone [MIBK]	108-10-1	11%	12%
Methylstyrene	25013-15-4	8%	8%
Naphthalene	91-20-3	12%	12%
2-Nitropropane	79-46-9	16%	17%
Pentachloroethane	76-01-7	11%	11%
Pentane	109-66-0	26%	25%

Analyte Name	CAS #	Water RSD	Solid RSD
2-Pentanone	107-87-9	15%	NA
2-Propanol [Isopropyl alcohol]	67-63-0	15%	13%
Propionitrile [Ethyl cyanide]	107-12-0	12%	11%
n-Propyl acetate	109-60-4	8%	16%
n-Propylbenzene	103-65-1	8%	9%
Styrene	100-42-5	8%	8%
1,1,1,2-Tetrachloroethane	630-20-6	8%	8%
1,1,2,2-Tetrachloroethane	79-34-5	9%	9%
Tetrachloroethene	127-18-4	9%	9%
Tetrahydrofuran	109-99-9	13%	13%
1,2,4,5-Tetramethylbenzene	95-93-2	11%	11%
Toluene	108-88-3	7%	7%
1,2,3-Trichlorobenzene	87-61-6	10%	11%
1,2,4-Trichlorobenzene	120-82-1	10%	11%
1,3,5-Trichlorobenzene	108-70-3	9%	10%
2,3,4-Trichlorobutene	2431-50-7	4%	NA
1,1,1-Trichloroethane	71-55-6	9%	9%
1,1,2-Trichloroethane	79-00-5	7%	7%
Trichloroethene (Trichloroethylene)	79-01-6	7%	8%
Trichlorofluoromethane [Freon-11]	75-69-4	12%	13%
1,2,3-Trichloropropane	96-18-4	8%	9%
1,1,1-Trichlorotrifluoroethane	354-58-5	9%	7%
Trifluorotoluene	98-08-8	6%	9%
1,1,2-Trichlorotrifluoroethane [Freon-113]	76-13-1	11%	12%
1,2,3-Trimethylbenzene	526-73-8	6%	6%
1,2,4-Trimethylbenzene	95-63-6	8%	8%
1,3,5-Trimethylbenzene	108-67-8	8%	9%
2,2,4-Trimethylpentane [Isooctane]	540-84-1	13%	14%
Vinyl acetate	108-05-4	15%	17%
Vinyl bromide	593-60-2	13%	7%
Vinyl chloride	75-01-4	14%	14%
Xylenes [total]	1330-20-7	7%	8%
m/p-Xylene [3/4-Xylene]	179601-23-1	7%	8%
o-Xylene	95-47-6	7%	8%

NA = Not Available

Table 3

4-BROMOFLUOROBENZENE (BFB) SUGGESTED CRITERIA*

m/z	Intensity (relative abundance)
95	50 to 200% of mass 174
96	5 to 9% of *m/z* 95 (5 to 15% when using H_2 carrier)
173	<2% of *m/z* 174
174	50 to 200% of mass 95
175	5 to 9% of *m/z* 174
176	95 to 105% of *m/z* 174
177	5 to 10% of *m/z* 176

* Criteria based on EPA Method 524.4 (Reference 17), with modified *m/z* 95 and *m/z* 174 abundance criteria

Table 4

GUIDANCE RESPONSE FACTORS CRITERIA FROM EPA CONTRACT LABORATORY PROGRAM

Analyte	RF
Acetone	0.01
Benzene	0.2
Bromochloromethane	0.1
Bromodichloromethane	0.3
Bromoform	0.1
Bromomethane	0.01
2-Butanone	0.01
Carbon disulfide	0.1
Carbon tetrachloride	0.1
Chlorobenzene	0.4
Chloroethane	0.01
Chloroform	0.3
Chloromethane	0.01
Cyclohexane	0.01
Dibromochloromethane	0.2
1,2-Dibromo-3-chloropropane	0.01
1,2-Dibromoethane (EDB, Ethylene dibromide)	0.2
1,2-Dichlorobenzene	0.6
1,3-Dichlorobenzene	0.5
1,4-Dichlorobenzene	0.6
Dichlorodifluoromethane	0.01
1,1-Dichloroethane	0.3
1,2-Dichloroethane	0.07
1,1-Dichloroethene (Vinylidene chloride)	0.06
cis-1,2-Dichloroethene	0.2
trans-1,2-Dichloroethene	0.1
1,2-Dichloropropane	0.2
cis-1,3-Dichloropropene	0.3
trans-1,3-Dichloropropene	0.3
Ethylbenzene	0.4
2-Hexanone	0.01
Isopropylbenzene	0.4
Methyl acetate	0.01
4-Methyl-2-pentanone	0.03
Methyl tert-butyl ether (MTBE)	0.1
Methylcyclohexane	0.05
Methylene chloride	0.01
Styrene	0.2
1,1,2,2-Tetrachloroethane	0.2
Tetrachloroethene	0.1

Toluene	0.3
1,2,3-Trichlorobenzene	0.4
1,2,4-Trichlorobenzene	0.4
1,1,1-Trichloroethane	0.05
1,1,2-Trichloroethane	0.2
1,1,2-Trichlorotrifluoroethane	0.05
Trichloroethene (Trichloroethylene)	0.2
Trichlorofluoromethane	0.01
Vinyl chloride	0.01
m,p-Xylene	0.2
o-Xylene	0.2

Values in this table are referenced from the CLP Statement of Work SOM 02.4. These response factors are provided as guidance only and are not intended to be a requirement. See Appendix B for additional information.

Table 5

RECOMMENDED QUANTITY OF EXTRACT FOR ANALYSIS OF HIGH CONCENTRATION SAMPLES

Approximate Concentration Range (µg/kg)	Volume of Extract[a]
500 - 10,000	100 µL
1,000 - 20,000	50 µL
5,000 - 100,000	10 µL
25,000 - 500,000	100 µL of 1/50 dilution[b]

Calculate appropriate dilution factor for concentrations exceeding this table.

[a] The volume of solvent added to 5 mL of water being purged should be kept constant. Therefore, add to the 5-mL syringe whatever volume of solvent is necessary to maintain a volume of 100 µL added to the syringe.

[b] Dilute an aliquot of the solvent extract and then take 100 µL for analysis.

Table 6

VOLATILE INTERNAL STANDARDS WITH CORRESPONDING ANALYTES ASSIGNED FOR QUANTITATION

FLUOROBENZENE	CHLOROBENZENE-d_5	1,4-DICHLOROBENZENE-d_4
Acetone	Benzene	p-Bromofluorobenzene (surrogate)
Acrylonitrile	Bromodichloromethane	Bromoform
Bromochloromethane	Carbon tetrachloride	*n*-Butylbenzene
Bromomethane	Chlorobenzene	*sec*-Butylbenzene
2-Butanone	Cyclohexane	*t*-Butylbenzene
Carbon disulfide	Dibromochloromethane	1,2-Dibromo-3-chloropropane
Chloroethane	1,2-Dibromoethane (EDB, Ethylene dibromide)	1,2-Dichlorobenzene
Chloroform	1,2-Dichloropropane	1,3-Dichlorobenzene
Chloromethane	*cis*-1,3-Dichloropropene	1,4-Dichlorobenzene
Dichlorodifluoromethane	*trans*-1,3-Dichloropropene	1,2-Dichlorobenzene-d_4 (surrogate)
1,1-Dichloroethane	Ethylbenzene	Hexachlorobutadiene
1,2-Dichloroethane	2-Hexanone	Isopropylbenzene
1,2-Dichloroethane-d_4 (surrogate)	Methyl cyclohexane	Isopropyltoluene
1,1-Dichloroethene (Vinylidene chloride)	4-Methyl-2-pentanone	Naphthalene
cis-1,2-Dichloroethene	Styrene	*n*-Propylbenzene
trans-1,2-Dichloroethene	1,1,1,2-Tetrachloroethane	1,2,3-Trichloropropane
1,4-Difluorobenzene (surrogate)	1,1,2,2-Tetrachloroethane	1,2,4-Trimethylbenzene
Freon 113	Tetrachloroethene	1,3,5-Trimethylbenzene
Methyl acetate	1,1,1-Trichloroethane	1,2,3-Trichlorobenzene
Methylene chloride	1,1,2-Trichloroethane	1,2,4-Trichlorobenzene
Methyl-*t*-butyl ether (MTBE)	Trichloroethene (Trichloroethylene)	
Trichlorofluoromethane	Toluene	
Vinyl chloride	Toluene-d_8 (surrogate)	
	m- + *p*-Xylene	
	o-Xylene	

Please note that this list is not exhaustive of all compounds found in the table in Sec 1.1 and are suggested IS associations only.

Table 7

SUMMARY OF QC CRITERIA FOR USE WITH 8260D[a]

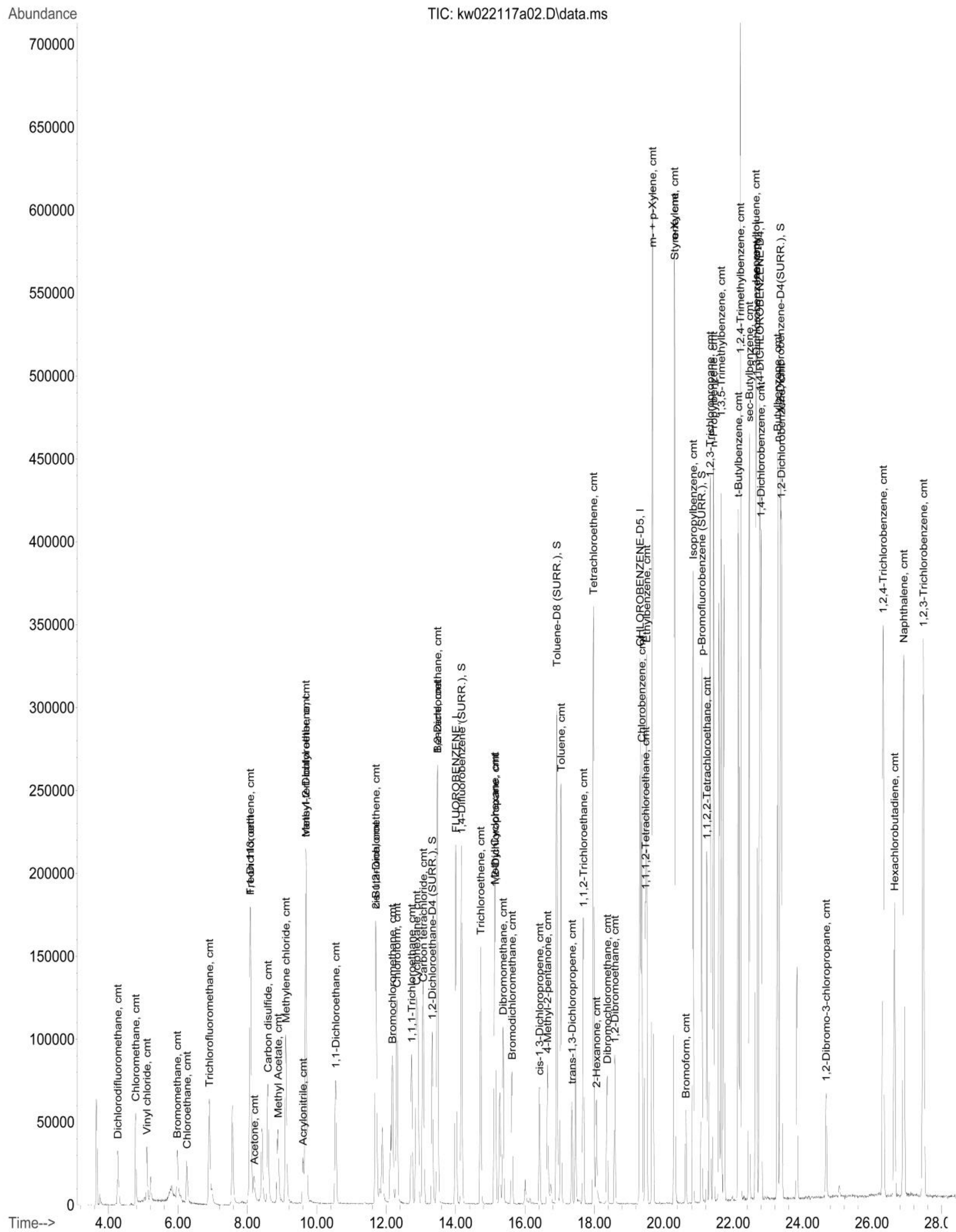
Quality Control Type	Minimum frequency	Specification	Suggested Acceptance Criteria
Instrument performance check (Secs. 9.3.1, 11.3.1)	Prior to initial calibration	Must be verified prior to initial calibration	Meet ion ratio criteria for reference compound: 4-Bromofluorobenzene (Table 3), or alternative documented criteria
Initial Calibration (ICAL) (Secs. 9.3.2, 11.3.2-11.3.5)	Prior to analyzing samples, and as needed if continuing performance criteria cannot be met	5 points minimum for RF and linear regressions, 6 points minimum for quadratic regressions; >90% of reported target analytes meet initial calibration criteria	For average response factor (RF) calibration model: ≤20% RSD of RFs; For linear or quadratic regression model: R≥0.995, R^2≥0.99; Independent of calibration model: LLOQ standard recalculation (refit) is within ±50% of true value if it is the low calibration point; All other standards within ±30% of true value; Or, relative standard error (RSE) ≤20% (Refer to Method 8000 and Reference 16 for calculation) See Method 8000 for additional criteria.
ICAL Verification (ICV) (Secs. 9.3.2, 11.3.6)	After each initial calibration, and prior to analyzing samples	Prepared from different source of target analytes than initial calibration standards	Calculated concentrations of target analytes are within ±30% of true value
Continuing Calibration Verification (CCV) (Secs. 9.3.3, 11.4)	Once every 12 hours	>80% of target analytes meet CCV criteria	Targets are ≤20% difference or drift; IS responses are within 50% to 200% of mid-point of ICAL or average of ICAL ISs; and RTs for ISs have not shifted >30 seconds relative to ICAL
Blanks (Secs. 9.5, 9.6.1)	One method blank per preparation batch of 20 or fewer samples; other blanks as needed	NA	Target analyte concentrations in blanks are <1/2 LLOQ, or ≤ 10% of concentration in field samples
Laboratory Control Sample (LCS) (Sec 9.6.2)	One per preparation batch of 20 or fewer samples	NA	Meets recovery criteria (CCV criteria may be used if LCS and CCV are identical)

Quality Control Type	Minimum frequency	Specification	Suggested Acceptance Criteria
Duplicates and Matrix Spikes (Secs. 9.6.3)	A duplicate and matrix spike, or matrix spike/matrix spike duplicate per preparation batch of 20 or fewer samples, provided adequate material is made available to the laboratory	NA	Meets performance-based or project-defined recovery criteria for matrix spikes; Meets relative % difference between measured concentrations in sample and laboratory duplicate or in matrix spike/matrix spike duplicate;
Surrogates (Secs. 9.7)	Added to each sample	NA	Meets performance-based recovery criteria established by the laboratory or criteria chosen for the project
Internal Standards (Secs. 9.8, 11.5.6)	Added to each sample	NA	IS response is within 50 - 200% of the response of the same IS in the midpoint ICAL standard (or average of ICAL) or most recent CCV
Qualitative Analyte Identification (Sec. 11.6.1)	Each target analyte	NA	RT in sample is within ±10 sec of RT in midpoint ICAL or CCV standard or within ±10 seconds relative to the shift of the associated IS (delta RT of the IS ± 10 seconds) Characteristic ion(s) are within ±30% of expected ion ratio in reference spectrum; or, match to reference library spectra ≥0.8 (only for full mass range acquisition modes)

[a] Default acceptance criteria; alternative criteria may be specified for a given application. Refer to Sec. 9 for more information.

Figure 1

EXAMPLE GAS CHROMATOGRAM OF VOLATILE ORGANICS [a]

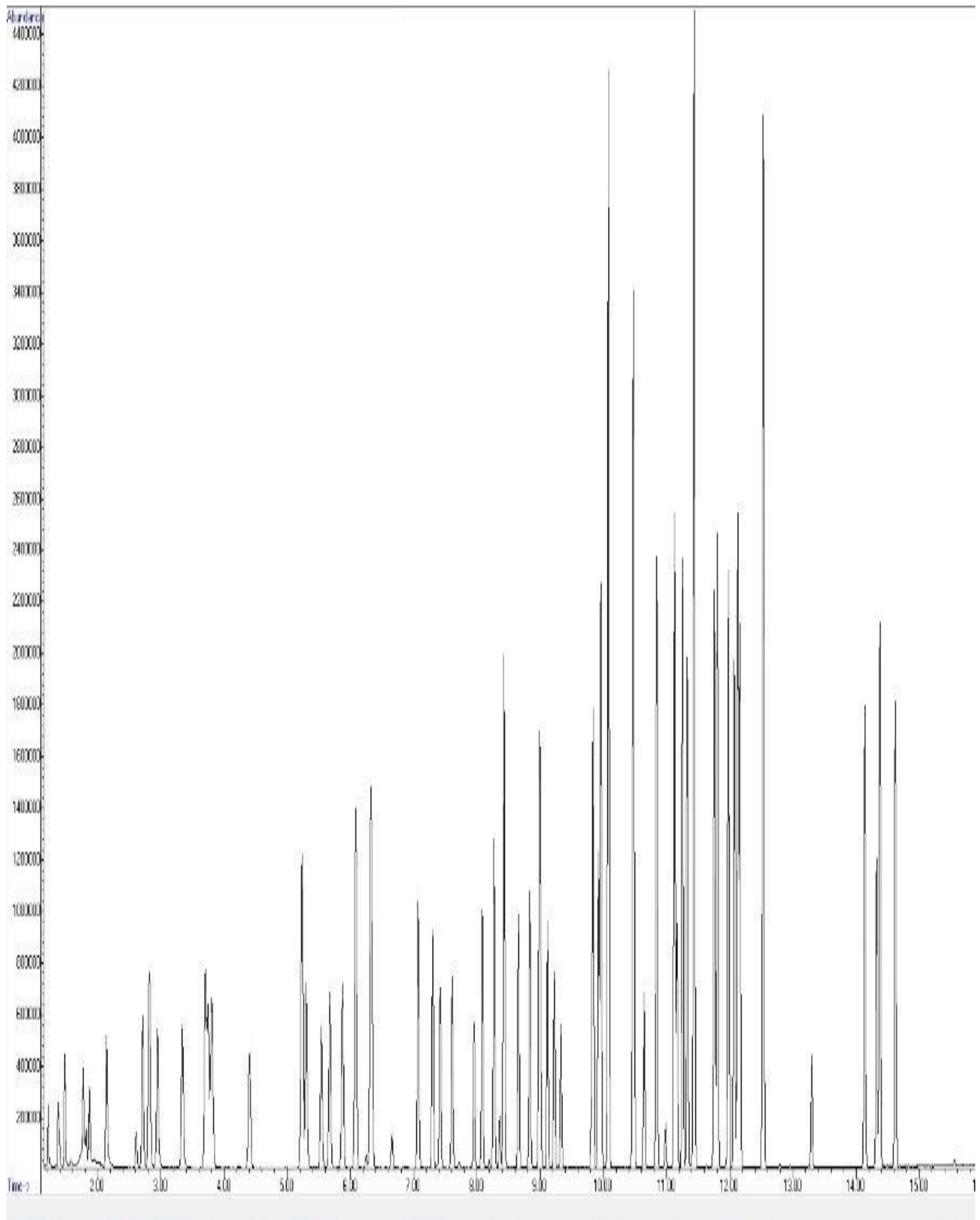


Figure

[a] Courtesy of EPA Region 10.

Figure 2

EXAMPLE GAS CHROMATOGRAM OF VOLATILE ORGANICS [a]



[a] Courtesy of EPA Region 5

1. Throughout the document the overall method formatting was updated for consistency with new SW-846 methods style guidance. The term mass was replaced with *m/z* to reflect what is actually being measured by the detector. Area or height was replaced with response. Language was added allowing the use of hydrogen gas as a carrier.

2. Section 1: The table (Sec. 1.1) was updated with new designations for performance (✓, *, etc.). Definitions of these symbols (Sec. 1.1) and expanded descriptions of compounds with known performance issues (Sec. 1.3) was added. Trichlorotrifluoroethane was split into two isomers: 1,1,2-Trichlorotrifluoroethane and 1,1,1-Trichlorotrifluoroethane.

3. Section 5: The designation MSDS (material safety data sheets) was updated to SDS (safety data sheets), as that is the correct term in the Global Harmonization System (GHS). A safety note was added regarding hydrogen.

4. Section 6: All vendors and product names were removed and replaced with generic terms (Sec. 6.2.1). MS acquisition rate was changed to minimum number of spectra per peak (Sec. 6.2.2.1). Subsections for tandem MS (Sec. 6.2.2.3) and SIM/CI (Sec. 6.2.2.4) were added.

5. Section 7: A paragraph was added about performing ICV with an alternate source (Sec. 7.11.3). Language was added regarding carrier gases to the reagents and standards section (Sec. 7.14).

6. Section 9: This section was completely updated and reorganized. New language and references were added from Method 8000. Sections on IDP (Sec. 9.4), blank language (Secs. 9.5 through 9.5.4), and LLOQ (Sec. 9.9) were updated and expanded. Significant revisions/additions were made to the blank section adding clarifying information about concentrations allowed in blanks (one half LLOQ), how blank concentration relates to sample concentration (<1/10), and some guidance for qualifying data. Information was added about the required frequency of LLOQ check standards (Sec. 9.9.1.2).

7. Section 11: This section was updated and reorganized. The chromatographic conditions were updated for commonly used columns (Secs. 11.2.1 through 11.2.5) and a set of conditions for hydrogen carrier gas was added (Sec. 11.2.6). The tuning criteria were updated for BFB for full scan analysis (Sec. 11.3 and Table 3), as well as other options, including SIM and/or CI analysis. Two notes were added (Sec. 11.3.1.2) regarding when each type of tune verification is appropriate. Tune verification frequency was also updated from once every 12 hours to once prior to ICAL. SIM and SRM guidance were updated (Sec. 11.3.3). A note was added regarding initial calibration curve fit when blank contamination is present and additional options for evaluation of calibration fit (Sec. 11.3.5). Updated language on ICV standards was added (Sec. 11.3.6). Clarified calibration verification frequency to allow for last initial calibration standard to be the start of 12-hour clock for samples analyzed after initial calibration (Sec. 11.4.1). Clarified that a blank is required after initial calibration and continuing calibration verification. Clarified that monitoring of ISs in CCVs is required. IS RT is now defined in absolute terms only (Sec. 11.4.4). Options to use mass spectral library searches to support qualitative identification were added (Sec. 11.6.1.3). Calculations for verifying chromatographic peak resolution were updated (Sec. 11.6.1.4). TIC interpretation language was revised (Sec. 11.6.2). Language was added regarding the analysis of TPH and GRO multicomponent mixes via total ion chromatogram (Sec. 11.7.5).

8. Section 13: Performance data listed previously in tables at the end of 8260C can now be

found at: https://www.epa.gov/hw-sw846/sw-846-test-method-8260d-volatile-organic-compounds-gas-chromatographymass-spectrometry.

9. Sections 14 and 15: The links to the listed safety documents were updated and replaced with the following links:
   a. http://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf and
   b. http://www.labsafetyinstitute.org/FreeDocs/WasteMgmt.pdf

10. Section 16.0: Updated Reference 1 and added Reference 13 for DOD data used to populate Table 2.

11. Table 1: New analytes with suggested ions were added.

12. Table 2: LLOQ limits were removed and replaced with 2012 DOD study data.

13. Table 3: BFB criteria were updated with new criteria from Method 524.3.

14. Table 4: Min RF table was renamed as guidance and a caution statement was added below the table. Compounds are listed alphabetically by compound name.

15. Table 6: Suggested IS associations were added. Compounds are listed alphabetically by compound name.

16. Table 7: Suggested QC criteria for use with Method 8260D were added.

17. Appendix B was added discussing the use of hydrogen as a carrier gas.

18. The SW-846 Workgroup conducted a thorough review of the use of the words "must" and "should" with regards to the requirements for the frequency and type of QC samples and the associated acceptance criteria for them in this method.

19. A table of contents was added and all graphics and tables in this method were updated to be 508 compliant.

B1.0 Guidance for Using Hydrogen Carrier Gas

B1.1 Hydrogen is an acceptable carrier gas to use for this analysis. However, the following modifications may be needed to make the analysis comparable to helium carrier gas:

B1.1.1 It is recommended that the highest purity (99.999% or better) hydrogen gas be used, such as from a generator or from high purity cylinders that will have minimal interferences present (e.g., hydrocarbons and water). Use of stainless steel tubing instead of copper tubing may increase the longevity of gas lines as older copper lines may become brittle over time with the use of hydrogen. MS ion source materials should be designed and approved for use with hydrogen. Contact the manufacturer of the MS to confirm the ion source is compatible with hydrogen.

Additionally, the pressure in the source should be reduced when hydrogen is used to prevent chemical ionization or other detrimental reactions from occurring. This may be done by the use of narrower bore columns (0.18 mm ID or smaller), reduction in the flow to the MS, and/or by the use of internal MS vacuum pumps (turbo pumps) with greater volumetric or pumping efficiency. Hydrogen may not be a suitable carrier gas for systems that have internal diffusion pumps.

B1.1.2 Use of hydrogen will clean (scrub) the metal surfaces of the analytical system of compounds that have adhered to the surface, generally hydrocarbons, and increase the background presence of these interferences. A bake-out of the system using high flows of hydrogen may decrease these interferences to a level that would not interfere with analysis. It is also recommended that new filters be installed on gas lines (or remove them altogether if gas purity is sufficient) to prevent the scrubbing of impurities from the filters.

B1.2 Use of hydrogen as the carrier gas may also reduce the responses of target analytes (i.e., approximately 2 - 5 times) as compared to helium. RF criteria listed in Table 4 were developed using helium carrier gas and are not appropriate for hydrogen carrier gas due to the reduced response of some analytes. If minimum RFs are used in evaluating the calibration, the laboratory should develop their own criteria or use published RFs from the instrument manufacturer. Reactivity of target analytes will vary with instrument conditions. As part of the demonstration of capability (DOC) process, evaluate target analytes for stability under the expected analytical conditions.

B1.3 Performance of some reactive target analytes may not be equivalent to performance using helium as a carrier. As with any method modification, all QC procedures listed in Sec. 9.0 of this method should be repeated and passed using hydrogen as the carrier gas prior to the analysis of samples. Use of alternate solvents for calibration standards and extracts would also require repeating these QC procedures prior to analysis of samples.

B1.4 Hydrogen gas is highly flammable and additional safety controls may be necessary to prevent explosive levels of gas from forming. This may be accomplished by connecting vent lines from the GC inlet and MS rough pump to exhaust systems in the laboratory and leak testing all gas line connections. The flow of hydrogen should also be turned off at the source prior to opening gas lines on the GC and prior to venting the MS (such as when maintenance is performed). The user should consult additional guidelines for the safe use of hydrogen from the instrument manufacturer prior to implementing its use.

METHOD 3585

WASTE DILUTION FOR VOLATILE ORGANICS

1.0 SCOPE AND APPLICATION

1.1 This method describes a solvent dilution of a non-aqueous waste sample prior to direct injection analysis. It is designed for use in conjunction with GC or GC/MS analysis of wastes that may contain organic chemicals at a concentration greater than 1 mg/kg and that are soluble in the dilution solvent. Method 3585 has adequate sensitivity to determine the regulatory concentrations of the Toxicity Characteristic (TC) Rule.

1.2 This method may be used with *n*-hexadecane for direct injection of target volatiles in oily matrices.

1.3 Use of a 1 - 2 µL injection of a 1:1 dilution can be used to provide detection limits of 0.5 ppm for volatile target analytes with a sensitive GC/MS.

1.4 This method is restricted to use by or under the supervision of trained analysts. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Highly contaminated or highly complex samples may be diluted prior to analysis for volatiles using direct injection.

2.2 One gram of sample is weighed into a capped tube or volumetric flask. The sample is diluted to 2.0 - 10.0 mL with *n*-hexadecane or other appropriate solvent.

2.3 Diluted samples are injected into the GC or GC/MS for analysis.

3.0 INTERFERENCES

3.1 Use of a direct injection procedure will result in considerable contamination of injection ports, injection port liners, GC columns, and detectors. A Pyrex® wool plug should be placed into the injection port liner and the liner should be changed after every 12 hours of sample analysis.

3.2 The solvent used for waste dilution may contain volatile contaminants that could interfere with analyses.

3.2.1 *n*-Hexadecane elutes after target volatiles. However, volatile impurities in n-hexadecane may interfere with analyses.

3.2.2 Each lot of *n*-hexadecane (or any other solvent used for dilution) must be analyzed for impurities prior to use.

3.3 The presence of methanol and other oxygenated solvents in samples may lead to baseline humps that interfere with qualitative and quantitative analysis of early eluting target analytes when direct injection is employed.

4.0 APPARATUS AND MATERIALS

4.1 Glass scintillation vials - At least 20-mL, with polytetrafluoroethylene (PTFE)- or aluminum foil-lined screw-cap, or equivalent.

4.2 Spatula - Stainless steel or PTFE.

4.3 Balance - Capable of weighing 100 g to the nearest 0.01 g.

4.4 Vials and caps - 2-mL, for GC autosampler.

4.5 Disposable pipets - Pasteur.

4.6 Test tube rack.

4.7 Pyrex® glass wool.

4.8 Volumetric flasks, Class A - 2- or 10-mL (optional).

4.9 Direct injection liner (HP catalogue #18740-80200 or equivalent) - Modify with a 1-cm plug of Pyrex® wool placed approximately 50-60 mm down the length of the injection port (towards the oven). A 0.53 mm ID column is mounted 1 cm into the liner from the oven side of the injection port, according to manufacturer's specifications. Figure 1 is an example of the placement of the glass wool plug in the liner.

mm

Figure 1 Modified Injector

5.0 REAGENTS

n-Hexadecane, *n*-$C_{16}H_{34}$ - Pesticide quality or equivalent.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

See the introductory material to this chapter, Organic Analytes, Sec. 4.1.

7.0 PROCEDURE

7.1 Samples consisting of multiple phases must be prepared by the phase separation method (Chapter Two) before extraction. The oil phase is prepared as outlined below. An aqueous phase is prepared and analyzed following the guidance in Method 5030.

7.2 The sample dilution may be performed in a 2- or 10-mL volumetric flask. If disposable glassware is preferred, the 10-dram vial may be calibrated for use. Pipet 2.0 mL of methanol into the vial and mark the bottom of the meniscus. Discard this solvent. Dry the vial.

7.3 Transfer approximately 1 g of the oil phase of the sample to a vial or volumetric flask (record weight to the nearest 0.1 g). Wipe the mouth of the vial with a tissue to remove any sample material. Cap the vial before proceeding with the next sample to avoid any cross-contamination.

7.4 Immediately dilute to volume with *n*-hexadecane or other appropriate solvent. The choice of solvents is dependent on the nature of the target analytes. *n*-Hexadecane is late eluting and, therefore, presents no solvent interference for the majority of volatile organics. An early eluting solvent, e.g., pentane or hexane, may be chosen if the target analytes are mid to late eluting.

7.5 Add surrogate spiking solution, if required, for the analytical method to be employed.

7.6 Cap and shake the sample for 2 minutes.

7.7 The extract is ready for analysis by GC Methods 8015 or 8021, or by GC/MS Method 8260.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One, Method 8000, and the analytical method to be employed, for specific quality control procedures.

8.2 Each time samples are prepared and analyzed, and when there is a change in reagents, a reagent blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. Any reagent blanks, matrix spike samples, or replicate samples should be subjected to exactly the same analytical procedures as those used on actual samples.

8.3 Standard quality assurance practices should be used with this method. Field duplicates should be collected to validate the precision of the sampling technique. Each analysis batch of 20 or fewer samples must contain: a reagent blank; either a matrix spike/matrix spike duplicate or a matrix spike and duplicate sample analysis; and a laboratory control sample, unless the determinative method provides other guidance.

8.4 Surrogates should be added to all samples when specified in the appropriate determinative method.

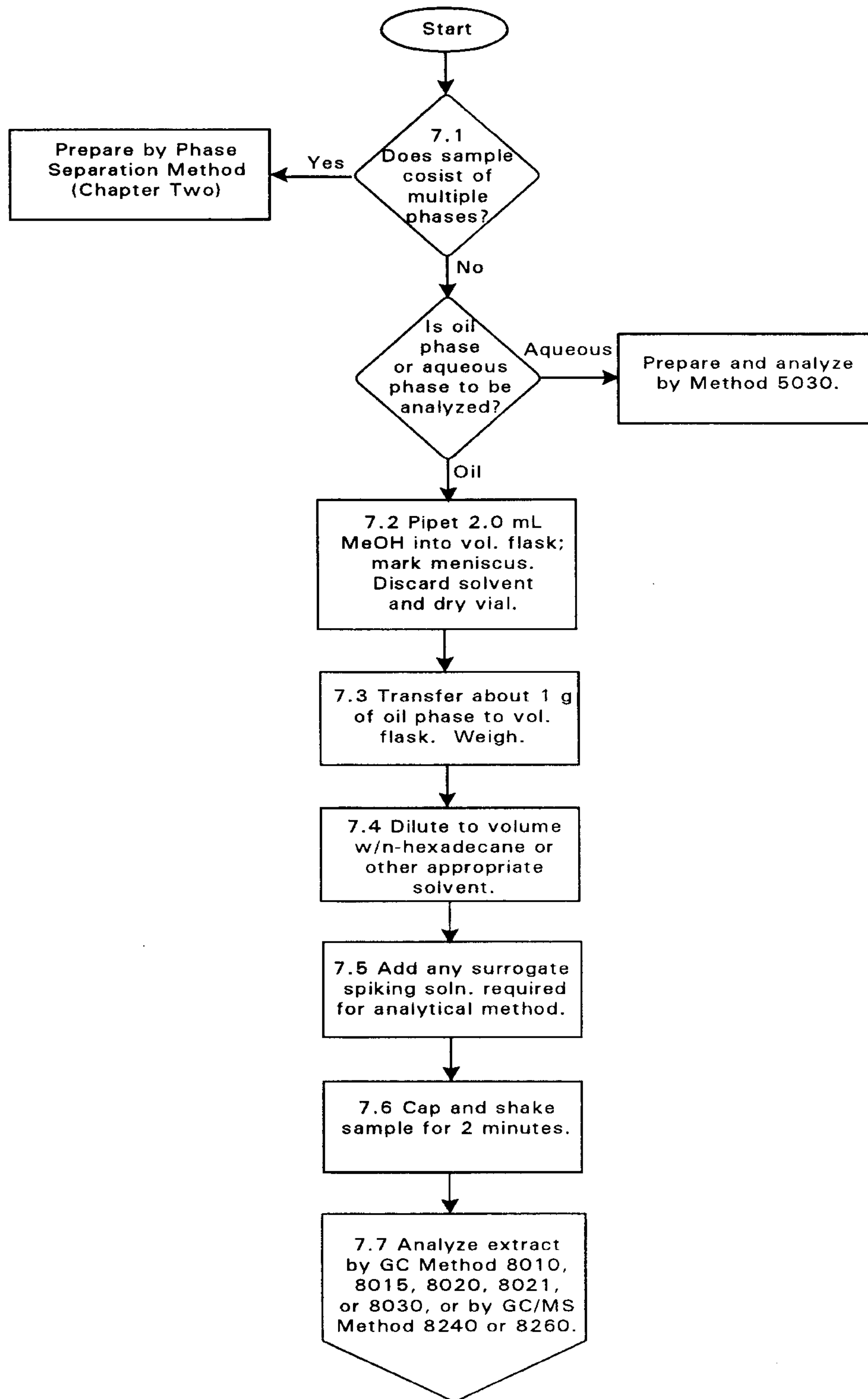
9.0 METHOD PERFORMANCE

Refer to the determinative methods for performance data.

10.0 REFERENCES

1. Marsden, P.J., Colby, B.N., and Helms, C.L., "Determining TCLP Volatiles at Regulatory Levels in Waste Oil", Proceedings of the Eighth Annual Waste Testing and Quality Assurance Symposium, July, 1992.

WASTE DILUTION FOR VOLATILE ORGANICS



METHOD 5021A

VOLATILE ORGANIC COMPOUNDS IN VARIOUS SAMPLE MATRICES USING EQUILIBRIUM HEADSPACE ANALYSIS

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute QC acceptance criteria or for the purpose of laboratory accreditation.

1.0 SCOPE AND APPLICATION

Please see Appendix A at the end of this document for a summary of changes from the previous version.

1.1 This method describes equilibrium-based static headspace preparation of volatile organic compounds (VOCs) in soil/sediment, solid waste, aqueous and water-miscible liquid samples for determination by gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS). This method is applicable to a wide range of organic compounds that have sufficiently high volatility to be effectively removed from samples using the described conditions. While the method is designed for use on samples containing low levels of VOCs or aqueous dilutions thereof to be analyzed by direct vapor partitioning, a solvent extraction and extract introduction procedure is also described for solid samples containing high concentrations of VOCs or for oily materials that may not be appropriate for the low level technique. This preparation method is intended to be combined with a determinative method such as Methods 8015, 8021 or 8260. This preparation method is appropriate for the compounds listed below, and it may also be appropriate for other VOCs included in the determinative method (*e.g.*, Sec. 1.1 of 8260), provided method performance is demonstrated to be acceptable for the intended use of the data.

Compound	CAS No.[a]	Response	Stability
Acetone	67-64-1	ws	hs
t-Amyl alcohol (TAA)	75-85-4	nd	hs
t-Amyl ethyl ether (TAEE)	919-94-8	nd	nd
t-Amyl methyl ether (TAME)	994-05-8	nd	hs
Benzene	71-43-2	c	hs
Bromochloromethane	74-97-5	p	hs
Bromodichloromethane	75-27-4	c	ms
Bromoform	75-25-2	p	hs
Bromomethane	74-83-9	c	hvs

Compound	CAS No.[a]	Response	Stability
t-Butyl alcohol (TBA)	75-65-0	ws	nd
Carbon tetrachloride	56-23-5	c	hvs
Chlorobenzene	108-90-7	c	hvs
Chloroethane	75-00-3	c	ms
Chloroform	67-66-3	c	hs
Chloromethane	74-87-3	c	hvs
Dibromochloromethane	124-48-1	p	nd
1,2-Dibromo-3-chloropropane	96-12-8	p	ms
1,2-Dibromoethane	106-93-4	p	hs
Dibromomethane	74-95-3	p	hs
1,2-Dichlorobenzene	95-50-1	c	hs
1,3-Dichlorobenzene	541-73-1	c	ms
1,4-Dichlorobenzene	106-46-7	c	ms
Dichlorodifluoromethane	75-71-8	c	hs
1,1-Dichloroethane	75-34-3	c	hs
1,2-Dichloroethane	107-06-2	p	hs
1,1-Dichloroethene	75-35-4	c	hvs
trans-1,2-Dichloroethene	156-60-5	c	ms
1,2-Dichloropropane	78-87-5	c	hs
Diisopropyl ether (DIPE)	108-20-3	c	hs
Ethanol	64-17-5	ws	nd
Ethylbenzene	100-41-4	c	hvs
Ethyl *tert*-butyl ether (ETBE)	637-92-3	c	hs
Hexachlorobutadiene	87-68-3	c	ms
Isopropanol	67-63-0	ws	nd
Methyl *tert*-butyl ether (MTBE)	1634-04-4	c	hs
Methylene chloride	75-09-2	c	hs
Naphthalene	91-20-3	p	ms
Styrene	100-42-5	c	hvs
1,1,1,2-Tetrachloroethane	630-20-6	c	hs
1,1,2,2-Tetrachloroethane	79-34-5	p	nd
Tetrachloroethene	127-18-4	c	ms
Toluene	108-88-3	c	hs
1,2,4-Trichlorobenzene	120-82-1	c	hs
1,1,1-Trichloroethane	71-55-6	c	ms
1,1,2-Trichloroethane	79-00-5	p	hs
Trichloroethene	79-01-6	c	ms
Trichlorofluoromethane	75-69-4	c	ls
1,2,3-Trichloropropane	96-18-4	p	ls
Vinyl chloride	75-01-4	c	hvs
o-Xylene	95-47-6	c	hvs
m-Xylene	108-38-3	c	hvs
p-Xylene	106-42-3	c	hvs
Gasoline range organics			

[a] Chemical Abstracts Service Registry Number

c = Response in reagent water is acceptable; similar response expected in matrix modifier solution (< 50% improvement).
p = Response in matrix modifier solution expected to improve >50% compared to reagent water; Use of matrix modifier is recommended.
ws = Highly water soluble analyte. Method sensitivity expected to be poorer than for other analytes due to poor partitioning into headspace; matrix modifier expected to be critical for acceptable method performance.
nd = Not determined
hs = High stability in preserved water samples (> 60 days). Longer holding times may be appropriate, see Method 5035, Appendix A, Table A.1 footnote and Ref. 47 for additional information
ms = Medium stability in preserved water samples (15 - 60 days). Longer holding times may be appropriate, see Method 5035, Appendix A, Table A.1 footnote and Ref. 47 for additional information
ls = Low stability in preserved water samples (< 14 days), analyses should be performed as soon as possible. May be degraded if acid preserved.
hvs = Highly variable stability depending on the sample matrix. Longer holding times may be appropriate, see Method 5035, Appendix A, Table A.1 footnote and Ref. 47 for additional information.

1.2 The following compounds may also be analyzed by this procedure or may be used as surrogates:

Compound	CAS No.[a]	Response	Stability
Bromobenzene	108-86-1	c	nd
n-Butylbenzene	104-51-8	c	nd
sec-Butylbenzene	135-98-8	c	nd
tert-Butylbenzene	98-06-6	c	nd
2-Chlorotoluene	95-49-8	c	nd
4-Chlorotoluene	106-43-4	c	nd
cis-1,2-Dichloroethene	156-59-4	c	hs
1,3-Dichloropropane	142-28-9	c	nd
2,2-Dichloropropane	590-20-7	c	nd
1,1-Dichloropropene	563-58-6	c	nd
Isopropylbenzene	98-82-8	c	nd
4-Isopropyltoluene	99-87-6	c	nd
n-Propylbenzene	103-65-1	c	nd
1,2,3-Trichlorobenzene	87-61-6	c	nd
α,α,α-Trifluorotoluene	98-08-8	nd	nd
1,2,4-Trimethylbenzene	95-63-6	c	nd
1,3,5-Trimethylbenzene	108-67-8	c	nd

[a] Chemical Abstracts Service Registry Number

1.3 In order to produce quantitative data with this technique, all of the quality control criteria described in the determinative method and/or Method 8000 should be met. Alternatively, this method may be utilized as a screening protocol. If used for screening, semi-quantitative or estimated sample results may be obtained with minimal calibration and quality control, such as a reagent blank and a single calibration standard.

As with any preparative method for volatiles, screening samples prior to low level analysis may help minimize problems associated with carryover contamination from samples that contain very high concentrations of volatiles above the calibration range of the determinative method. In addition, because removing a sample aliquot from a container may compromise the integrity of the sample, multiple sample aliquots should be collected to allow for screening and re-analysis.

1.4 In order to accommodate analysis of a variety of sample matrices and VOCs, a matrix modifier (Sec. 7.7) is generally recommended to be used with this method. The matrix modifier is a water soluble salt solution that is added to each sample and standard vial prior to analysis. The matrix modifier solution acts to increase the VOCs mass transfer into the headspace of the vial. The principal benefits of using the matrix modifier are:

1) better response and reproducibility of the VOCs that do not otherwise partition efficiently into the headspace of the vial from the aqueous phase (identified with 'p' or 'ws' in the response column in the table in Sec. 1.1); and

2) less potential for measurement bias resulting from aqueous activity differences between standards and samples.

Measurement bias results from VOCs partitioning into the vial headspace differently in a sample than in the calibration standards. Some potential sources of measurement bias and the anticipated effects of the matrix modifier on these sources of bias are described below.

1.4.1 Aqueous field samples containing high dissolved solute concentrations:

At higher solute concentrations substantially larger fractions of some VOCs partition into the headspace leading to high bias in the determined concentration. The VOCs most prone to high bias measurement at higher dissolved solute concentrations are also the VOCs whose responses are most substantially improved in the matrix modifier solution relative to reagent water (identified with 'p' or 'ws' in the response column in the tables in Sec. 1.1). The VOCs identified with 'c' in the response column in the analytes table in Secs. 1.1 and 1.2 are not as subject to this source of measurement bias. The matrix modifier is used to normalize the solute concentration between samples and calibration standards, thereby minimizing this source of bias.

1.4.2 Aqueous field samples containing water miscible organic component:

The presence of a water miscible organic component (e.g., cosolvent or surfactant) may result in low bias measurement of VOCs with high octanol-water partitioning coefficients (e.g., C3 and C4 alkylbenzenes, trichlorobenzenes and naphthalene), while recovery of the lighter and more highly water soluble VOCs is unlikely to be strongly affected unless the proportion of the water miscible organic component in the sample is high. The matrix modifier helps improve the recovery of VOCs whose partitioning into the headspace is most strongly affected by this source of measurement bias.

1.4.3 Field samples containing a water immiscible component:

For samples with a separate water immiscible phase, partitioning of VOCs into the headspace competes with the water immiscible phase. While addition of the matrix modifier has a favorable effect on partitioning of VOCs into the headspace from the aqueous phase, it may also increase partitioning into the water immiscible phase(s) (e.g., soils with >1% organic matter, oily materials), potentially exaggerating matrix effects relative to the calibration standards. This matrix effect is more pronounced for VOCs with higher octanol-water partitioning coefficients when the matrix modifier is used for the analysis. Recovery of the lighter and more water soluble VOCs is expected to be less affected.

For complex samples, more than one of these types of matrix effects may be relevant, and a compromise may have to be made for data quality of some analytes in order to obtain reliable data for the analytes deemed most critical for the project. For simple sample matrices and VOCs

not expected to subject to measurement bias (*e.g.*, analysis of BTEX and other alkylbenzenes in surface water samples) the matrix modifier solution may be omitted.

1.5 This method, in conjunction with determinative Method 8015 (GC/FID), may be used for analysis of the aliphatic hydrocarbon fraction in the light ends of petroleum hydrocarbons, *e.g.*, gasoline. For the aromatic fraction (BTEX), use this method and Method 8021 (GC/PID). A total determinative analysis of gasoline and other volatile petroleum hydrocarbon fractions may be obtained using Method 8021 in series with Method 8015. If MS detection is desired for these target analytes, Method 8260 (Volatile Organic Chemicals by GC/MS) may be used.

1.6 Measurements of VOCs using this method may be subject to bias from several sources, including differences in partitioning of VOCs between the aqueous phase and headspace in samples relative to standards, differences in headspace volume in samples relative to standards, and adsorption of VOCs to surfaces or absorption into compatible phases (*e.g.*, soil organic matter). Measurement bias is monitored through internal standard, surrogate, and matrix spike recovery when appropriate for the project and determinative method. Use of the matrix modifier (Sec. 7.7) will help minimize measurement bias resulting from differences in partitioning behavior of VOCs in samples relative to standards. Measurement bias resulting from adding solid material to the vial, which changes the headspace volume in the sample relative to the calibration standards, is expected to be negligible as long as the volume of material is small relative to the headspace volume. The magnitude of this bias may be reduced by adding a similar volume of solid organic-free control material to calibration standards as the volume of the bulk material being tested. Measurement bias related to sorption of VOCs to solid samples with fine particle size distributions and/or significant organic content may be substantial. The magnitude of this bias may be reduced by analyzing a smaller amount of material or by solvent extraction (Sec. 11.4).

1.7 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (*e.g.*, Methods 3500, 3600, 5000, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.8 This method is restricted to use by, or under supervision of, appropriately experienced and trained analysts for volatile organic analysis in general and specifically the use of equilibrium headspace devices interfaced to the determinative method selected by the analyst. Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Sample collection and headspace vial preparation

2.1.1 Water samples – A 40-mL volatile organic analyte (VOA) vial is filled to capacity and capped so no headspace remains in the vial. The water sample may be preserved at the time of sampling by addition of a chemical preservative (*e.g.*, hydrochloric acid solution, solid sodium bisulfate or solid trisodium phosphate) to the vial. At the laboratory, that vial is sub-sampled into a headspace vial, and internal standards and surrogates are added, if used. The matrix modifying solution (Sec. 7.7) should be added to the headspace vial during subsampling if used for the analysis.

2.1.2 Low concentration soil samples -- Approximately 2 g of soil is collected with an appropriately sized coring tool and placed in a pre-weighed crimp seal or screw top glass headspace vial, and then the vial is sealed. Depending on the analytes of interest, the soil sample may be preserved by addition of a pH modifying chemical preservative (*e.g.,* sodium bisulfate, trisodium phosphate) prior to sealing the sample vial, and the matrix modifying solution (Sec. 7.7) should also be added prior to capping the vial if used for the analysis. Sec. 8.3.3 also describes the use of a sealable coring device as an alternative sampling technique, which may simplify collection and handling of soils in the field.

Surrogates and internal standards may be added to the vials during sampling or at the laboratory. If the matrix modifying solution is used for the analysis and was not added to sample vials in the field, it should be added when any surrogates and internal standards are added at the laboratory. Adding the matrix modifying solution or reagent water to a vial after adding the sample may cause loss of gas phase VOCs from the container due to displacement of a portion of the vial headspace. Adding the matrix modifying solution (Sec. 7.7) to the vial prior to adding the sample and sealing quickly will help to limit loss of VOCs from the sample container and maintain sample representativeness.

NOTE: The choice of chemical preservative(s) will depend on the VOCs that will be measured in the samples and to some extent on the sample matrix. The matrix modifying solution acts as a chemical preservative, but it does not otherwise alter the sample pH and may not protect against degradation of some classes of VOCs, including hydrolysis of ethers or dehydrohalogenation of chlorinated aliphatics (Sec. 4.7). Sodium bisulfate has also been identified as inappropriate for use as a preservative for calcareous soils, which may off-gas CO_2 when exposed to acid due to chemical reaction with any carbonate salts, which may cause loss of VOCs from the container or build up pressure once the container is sealed, potentially leading to rupture.

2.1.3 High concentration soils or other solid materials – A representative portion of soil is collected with an appropriately sized coring tool and placed in a pre-weighed glass VOA vial, and then the vial is sealed. The soil sample may be preserved by addition of extraction solvent (*e.g.,* methanol) at the time of sampling or upon receipt by the laboratory. At the laboratory, the methanol extract is then diluted with the matrix used for the calibration standards (organic-free reagent water or the matrix modifying solution) and analyzed as an aqueous sample. Sec. 8.3.3 also describes the use of an air-tight sealable coring device as an alternative sample collection technique that may be useful, and Sec. A.6 of SW-846 method 5035A provides additional information pertaining to methanol extraction of soils.

NOTE: Surrogate compounds may either be spiked into the solvent at the time of extraction or into reagent water containing an aliquot of the extract prior to analysis. Since the surrogate recovery data from these two options provides assurances of either extraction or analytical efficiencies, the decision as to

when the surrogates are added depends on what questions need to be answered for a given sample matrix and the intended uses of the data.

2.2 For soil samples, additional aliquot(s) are collected in VOA vials for dry weight determination.

2.3 In the laboratory, the vials are rotated to allow for diffusion of internal standards and surrogates throughout the matrix. The vials are placed in the autosampler carousel of the headspace analyzer and maintained at room temperature. Approximately 1 hr prior to analysis, the individual vials are moved to a heated zone and mechanically agitated while the elevated temperature is maintained, allowing the VOCs to equilibrate between the headspace, liquid and any solid phases in the vial.

2.4 The autosampler then pressurizes the vial with helium and forces a portion of the headspace gas mixture into the gas chromatograph through a heated transfer line, either passing through the GC inlet or directly connected to the analytical column via an inert, low dead volume connector.

2.5 Determinative analysis is performed using the appropriate GC or GC/MS method. Any chemical preservative and matrix modifier added to the field samples should also be added to the calibration standards and other QC samples.

3.0 DEFINITIONS

Refer to Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to Chapter Four for general guidance on the cleaning of glassware. Also refer to the determinative methods to be used for information regarding potential interferences.

4.2 Volatile organic analyses are subject to major interference problems because of the prevalence of volatile organics in a laboratory. See Method 5000 for common problems and precautions to be followed.

4.3 Samples can be contaminated by diffusion of volatile organics (particularly methylene chloride and fluorocarbons) through the septum seal of the sample vial during shipment and storage. A trip blank, prepared from an appropriate organic-free matrix and sample container and carried through sampling and handling protocols, serves as a check on such contamination.

4.4 The sample matrix itself can cause severe interferences by one of several processes or a combination of these processes. These include, but are not necessarily limited to, the absorption potential of the soil, the biological activity of the soil, and the actual composition of the soil. Soils high in organic matter or oily material and organic sludge wastes inhibit the

partitioning of the volatile target analytes into the headspace. Therefore, analyte recovery by direct vapor partitioning may be low and will depend on the properties of the particular chemical. This matrix effect can be difficult, if not impossible, to overcome. It is recommended that surrogates or additional deuterated compounds (for GC/MS methods) be added to a matrix and analyzed to determine the percent recovery of these compounds. The calculated percent recovery can give some indication of the degree of the matrix effect, but not necessarily correct for it. Alternatively, the use of the high-concentration procedure in this method should minimize the problem with oily waste and other organic sludge wastes.

4.5 Contamination by carryover can occur whenever high concentration and low concentration samples are analyzed sequentially. Where practical, samples with unusually high concentrations of analytes should be followed by an analysis of one or more method blanks or instrument blanks to check for cross-contamination. If the target compounds present in an unusually concentrated sample are also found to be present in subsequent samples, the analyst must demonstrate that the compounds are not affected by carryover contamination. Conversely, if those target compounds are not present in the subsequent sample, then the analysis of a blank is not necessary.

4.6 The laboratory where volatiles analysis is performed should be free of any solvents that may interfere with the analysis. Special precautions must be taken when analyzing for methylene chloride. The analytical and sample storage areas should be isolated from all atmospheric sources of methylene chloride. Otherwise, random background levels can result. Since methylene chloride can permeate through polytetrafluoroethylene (PTFE) tubing, all GC carrier gas lines and purge gas plumbing should be constructed of stainless steel or copper tubing. Laboratory workers' clothing previously exposed to methylene chloride fumes during common liquid/liquid extraction procedures can contribute to sample contamination. The presence of other organic solvents in the laboratory where volatile organics are analyzed can also lead to random background levels, and the same precautions must be taken.

4.7 Ethers in acidic samples (*i.e.,* samples with a pH < 7) will hydrolyze at the higher temperatures used in this method. As such, basic preservatives should be used if the target analytes are ethers or the alcohols that those ethers would form if hydrolyzed. Strong bases may catalyze substitution and elimination reactions that can occur if halogenated compounds are present. Halogenated aliphatic VOCs are particularly susceptible to dehydrohalogenation reactions in neutral to basic conditions at elevated temperature such as with a heated sample preparation procedure as is described here. Accordingly, acidic preservatives may be necessary to prevent dehydrohalogenation if halogenated aliphatic VOCs are analytes of interest or their presence is suspected and their transformation products are of interest. Acetone has also been observed to form in high organic content soils preserved with sodium bisulfate (Sec. A.8 in the Appendix of method 5035A provides more information). The chemical reactivity introduced by the preservative should be monitored by analyzing a matrix spike of a field sample with each batch. The spiking solution should contain all analytes which the client intends to monitor.

5.0 SAFETY

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals included in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

This section does not list all common laboratory glassware (e.g., beakers and flasks) that might be used.

6.1 Headspace containers - Clear glass, 22-mL vials equipped with PTFE-lined septa that are compatible with the analytical system. Vials of other sizes may be employed, provided that they can be hermetically sealed and equipped with suitable septa. Ideally, the vials and septa should have a uniform tare weight. The septa should be unpunctured, as piercing the PTFE face may allow target analytes to diffuse into and adsorb to the silicone backing material. New, disposable vials may be used without pretreatment provided they are demonstrated to be clean through method blank analysis. Store the vials in an area free of organic solvents. If vials are suspected of being a source of contamination, first wash the vials in a detergent solution, then thoroughly rinse with tap water followed by distilled water, and finally dry the vials in an oven at 105 °C for 1 hour. Allow vials to cool prior to use.

6.2 Headspace system - The operating conditions listed in Sec. 11.0 are those selected for the equipment used in developing this method. See Reference #1 in Sec. 16 for more detail. Other equipment and conditions may be employed, provided that the laboratory demonstrates performance for the analytes of interest using the determinative method appropriate for the intended application. The system used must meet the following specifications:

6.2.1 The system must be capable of holding samples at elevated temperatures and establishing a reproducible equilibrium between a wide variety of sample types and the headspace.

6.2.2 The system must be capable of accurately transferring a representative portion of the headspace into a gas chromatograph fitted with a capillary column without adversely affecting the chromatography or the detector.

6.3 Field sampling equipment

6.3.1 Water samples - Clear or amber 40 mL volatile organic analysis (VOA) vials with screw-cap PTFE lined vials.

6.3.2 Soil samples

6.3.2.1 A soil sampler which delivers at least 2 g of soil is necessary, *e.g.*, Purge and Trap Soil Sampler Model 3780SPT (Associated Design and Manufacturing Company, 814 North Henry Street, Alexandria, VA 22314), or equivalent.

6.3.2.2 An automatic syringe or bottle top dispenser calibrated to deliver a 10.0 mL liquid volume.

6.3.2.3 Crimping tool for headspace vials - If using screw-top vials, this is not needed.

6.3.2.4 VOA vials (22, 40 or 60 mL) with PTFE faced septa and crimp-seal caps or screw-top caps. These vials will be used for sample screening, high concentration analysis (if needed) and dry weight determination.

6.3.2.5 Sealable, air-tight coring device – A soil coring device with an internal volume appropriate for approximately 2 g of sample for direct vapor partitioning analysis, or other size as appropriate for high level analysis, equipped with an o-ring seal or equivalent air-tight sealing mechanism, constructed of materials that will not absorb or react with the target chemicals of interest and with a cross-sectional diameter appropriate for a VOA vial compatible with the headspace analyzer or for use with methanol extraction.

6.4 Miscellaneous equipment

6.4.1 For the preparation of blanks, standards and water samples, it is necessary to have the crimping tool addressed in 6.3.2.3 available in the laboratory.

6.4.2 Graduated microsyringes for standard preparation and for addition of internal standard and surrogate spiking solutions.

6.4.3 5-mL glass hypodermic syringes with Luer-Lok™ tip (other sizes are acceptable depending on sample volume used).

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade chemicals must be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. Reagents should be stored in glass to prevent the leaching of contaminants from plastic containers.

7.2 Organic-free reagent water - Reagent water must be interference free. All references to water in this method refer to organic-free reagent water, unless otherwise specified.

7.3 Methanol - Pesticide quality or equivalent. Store away from other solvents. Purchase in small quantities (1 Liter size or less) to minimize shelf life to reduce potential for contamination.

7.4 See the determinative method and Method 5000 for guidance on the preparation of stock standards and a secondary standard for internal standards, calibration standards, and surrogates.

7.4.1 Calibration spiking solutions - Prepare five or more spiking solutions in methanol or water that contain all the target analytes. The concentrations of the calibration solutions should be such that the addition of 1.0 µL of each to the headspace vials will bracket the analytical range of the detector. Alternatively, calibration standards may be prepared by adding different volumes of one or more stock solutions provided that the

linearity of the calibration is not affected by the methanol content. For analysis of methanol extracts, it may be appropriate to calibrate surrogates at multiple concentration levels as well to demonstrate calibration linearity at the surrogate level measured in diluted extracts.

7.4.2 Internal and surrogate standards – Follow the recommendations of the determinative method for the selection of internal and surrogate standards. Selection and use of surrogates with physical properties similar to the classes of target analytes that are of interest for the project will provide more meaningful sample-specific quality assurance information. A concentration of 20 mg/L in methanol for both internal and surrogate standards may be used for spiking each sample. The concentration may vary depending on the relative sensitivity of the detector used in the determinative method. If determination is by GC, external standard calibration may be preferred and the internal standard omitted.

7.5 Blank preparation - Transfer 10.0 mL of matrix modifying solution (Sec. 7.7) or reagent water to a sample vial. Inject the necessary amounts of internal standards and surrogate compounds under the surface of the water in the headspace vial, and seal the vial. Place in the autosampler and analyze in the same manner as an unknown sample. Any chemical preservative and/or matrix modifier added to the field samples must also be included in the blank(s).

7.6 Preparation of calibration standards - Prepare calibration standards in the same manner as blanks (Sec. 7.5), adding the standard spiking solution(s) prepared in Sec. 7.4.1 in the same manner that internal standards and surrogates are added. Any chemical preservative and/or matrix modifier added to the field samples should also be included in the calibration standards.

7.7 Preparation of matrix-modifying solution - Add 180 g of ACS-grade sodium chloride (NaCl) to 500 mL of reagent water. Mix well until all components are dissolved. Other water soluble salts may be appropriate. The matrix modifier solution should not affect the pH of the sample to the extent that preservation or analyte stability is compromised. Analyze a 10.0-mL portion from each batch according to Sec. 7.5 to verify that the solution is free of contaminants. Store the prepared matrix-modifying solution in a sealed bottle in an area free of organic chemicals at ≤6 °C.

CAUTION: The matrix modifying solution may not be appropriate for analysis of some VOCs in soil samples having high organic matter content.

7.8 Preparation of chemical preservative for low level (vapor partitioning) analysis - The preservative should be chosen based on the analytes of interest and should be mixed with the sample at the time of sampling.

7.8.1 If a basic preservative is chosen, 100 mg of ACS-grade trisodium phosphate dodecahydrate (TSP; $Na_3PO_4 \cdot 12H_2O$) should be added to either a 22-mL headspace vial or a 40-mL water sample vial to raise the pH above 10.

7.8.2 If an acidic preservative is chosen, 2-3 drops of 6N hydrochloric acid (HCl) should be added to a 40-mL water sample vial. The HCl solution should be prepared by the 1:1 dilution of ACS-grade concentrated HCl. For acid preservation of a soil sample, 1 g of solid, ACS-grade sodium bisulfate ($NaHSO_4$) should be added to each 22-mL vial.

CAUTION: If samples containing MTBE, TAME, ETBE or other fuel ethers have been acid preserved with either sodium bisulfate or hydrochloric acid, these samples must be adjusted to pH >10 with trisodium phosphate

dodecahydrate (TSP) (Sec. 7.8.1) prior to initiation of the headspace analysis.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining an appropriate plan for sample collection, preservation and storage prior to sample collection and analysis.

8.1 Refer to Chapter Four and Method 5035A or general sample collection information. All samples should be stored in capped vials at ≤6 °C in an area free of solvent fumes If any evidence of leakage is found, the sample can be considered corrupted and should be discarded.

Pre-testing of a representative soil or aqueous sample, prior to collection, with acid or bisulfate may show effervescence if carbonaceous materials are present. If bubbling occurs during chemical preservation, an increased potential for loss of volatile constituents exists and samples should therefore be collected without preserving with acid or bisulfate.

8.2 Water samples - Fill the 40-mL vial and, according to the analyte list to be analyzed, chemically preserve the sample (Sec. 7.8) as necessary. Ensure that there is no headspace in the vial and seal it. At least two vials should be collected per sample and more may be necessary for duplicate and MS/MSD analyses, if desired. Transfer of the sample into a headspace vial and the addition of the matrix modifier and standards should be performed at the laboratory.

In general, liquid samples should be poured into the vial without introducing any air bubbles into the sample as the vial is being filled. Should bubbling occur as a result of violent pouring, the sample should be poured out and the vial refilled. The vials should be completely filled at the time of sampling, so that when the septum cap is fitted and sealed, and the vial inverted, no headspace is visible. The sample should be hermetically sealed in the vial at the time of sampling, and not opened prior to analysis to preserve its integrity.

Due to differing solubility and diffusion properties of gases in liquid matrices at different temperatures, it is possible for the sample to generate some headspace during storage. This headspace will appear in the form of micro bubbles and should not invalidate a sample for volatiles analysis. The diameter of any bubble caused by degassing upon cooling the sample should not exceed 5 - 6 mm. When a bubble is present, also inspect the cap and septum to ensure that a proper seal was made at the time of sampling. The presence of a macro bubble in a sample vial generally indicates either improper sampling technique or a source of gas evolution within the sample. The latter case is usually accompanied by a buildup of pressure within the vial, (e.g. carbonate-containing samples preserved with acid). Studies conducted by the USEPA (EMSL-Ci, unpublished data) indicate that "pea-sized" bubbles (i.e., bubbles not exceeding 1/4 inch or 6 mm in diameter) did not adversely affect volatiles data. These bubbles were generally encountered in wastewater samples, which are more susceptible to variations in gas solubility than are groundwater samples.

8.3 Soil samples - Three alternative procedures are presented below for collection of soil samples in headspace sample vials. Sec. A.7 in the appendix of method 5035A describes some additional alternatives that may also be appropriate. The choice between these alternatives should be based on knowledge of the field conditions, the organic carbon content of

the soil, the specific volatile analytes and concentration levels of interest, and the intended use of the analytical results. For low level analysis by direct vapor partitioning, 3 or 4 replicate samples should be collected from each sampling point to allow for reanalysis, while duplicate samples may be sufficient for high level analysis because the solvent extract can be diluted and reanalyzed. Additional sample replicates should also be collected for duplicate and MS/MSD analyses, as well as separate portions for dry weight determination. If samples will be analyzed by the low level method but have the potential to contain high levels of VOCs, samples may be collected for both low level and high level analysis. This is due to the difficulty of diluting samples prepared for low level analysis once they are sealed in the vials.

8.3.1 Sampling directly into prepared headspace vials for low level analysis:

Soil may be sampled by addition to a prepared vial that contains 10.0 mL of matrix modifier or reagent water, plus any necessary pH altering chemical preservative. The preservative and matrix modifier or water are added to the vial prior to sampling in order to prevent displacing a portion of the headspace from the vial, along with any associated VOCs . The matrix modifying solution has the additional benefits of reducing the biodegradation potential of the sample matrix and increasing partitioning of the VOCs into the vial headspace from water. Problems related to contamination of the aqueous solution in a field sampling situation and incorrect measurement and transfer into the sample vials can be minimized by adding it to the vials at the laboratory and sealing them prior to sending them to the field. Samples should be obtained and transferred to a vial rapidly after sampling (<10 seconds) to minimize volatilization losses. In order to estimate the sample mass added, the vial, cap and any added solutions should be tared and the masses recorded prior to and after adding a soil sample to the vial. If the vials were not prepared in the laboratory prior to sampling, the analyzing laboratory must be made aware of the identities and amounts of any reagents added to each vial in the field.

8.3.1.1 Use standard glass headspace vials with PTFE faced septa.

8.3.1.2 Using the soil sampler (Sec. 6.3.2.1), add 2-3 cm (approximately 2 g) of the soil sample to a tared headspace vial containing 10.0 mL of matrix modifier or reagent water and any pH modifying chemical preservative used. The samples should be introduced into the vials gently to reduce agitation which might drive off volatile compounds. Seal immediately with the PTFE side of the septum facing toward the sample.

8.3.2 Sampling directly into empty or prepared headspace vials for high level analysis:

If high concentrations of VOCs are expected (greater than 200 µg/kg), collection of the sample in an empty headspace vial or a vial containing methanol is appropriate for use with the high concentration procedure described in Sec. 11.4**.**

8.3.2.1 Use standard 22-mL crimp-cap or screw-top glass headspace vials with PTFE faced septa (other vials may be used, as described in Sec. 6.1).

8.3.2.2 Using the soil sampler (Sec. 6.3.2.1), add 2-3 cm (approximately 2 g) of the soil sample to a headspace vial and seal immediately with the PTFE side of the septum facing toward the sample. The samples should be obtained and transferred to a vial rapidly after sampling (<10 seconds) to minimize volatilization losses, and they should be introduced into the vials gently to reduce agitation which might drive off volatile compounds. If methanol is added to the vial

prior to the sample, the vial, cap and methanol should be tared and the masses recorded prior to and after adding a soil sample to the vial. The recorded mass should be checked by the analyzing laboratory to verify that solvent was not lost during shipping and/or storage.

8.3.3 Sampling with a sealable, air-tight coring device for low or high level analysis:

For cohesive soils, soil samples can be taken in appropriately sized air-tight sealable coring devices for refrigeration and shipping to the laboratory, where the samples are further preserved or immediately prepared for analysis.

8.3.3.1 Insert a clean coring device into a fresh surface for sample collection and ensure that no air is trapped between the coring tool and the sample. The volume of material collected should not cause excessive stress on the coring tool during intrusion into the material. Just before capping, a visual inspection of the lip and threads of the sample vessel should be made and any foreign debris should be wiped clean, allowing an airtight seal to form.

8.3.3.2 Upon laboratory receipt, the soil plug in each sealable coring device is extruded into individual tared headspace VOA vials containing the appropriate solution (either matrix modifier or reagent water for low level analysis, with pH modifying preservative as appropriate, or methanol for high level analysis). The coring device must fit into the mouth of the headspace vial or other VOA vial into which the sample is extruded, or losses of VOCs will result. In order to estimate the sample mass added, the vial, cap and any added solutions should be tared and the mass recorded prior to and after adding a soil sample to the vial.

8.4 Field blanks should be prepared, regardless of which alternative is employed for soil sample collection. If the matrix modifying solution is not added in the field, then the field blank(s) should be prepared by adding any reagents used in the field (*e.g.*, 10.0 mL of organic-free reagent water, methanol, or matrix modifying solution, plus any other chemical preservatives) to a clean vial and immediately sealing the vial.

8.5 Sample storage

8.5.1 Samples should be stored at ≤6 °C until analysis in order to limit diffusion of the analytes out of the water, reduce the ability of the analytes to react with the glass walls of the sampling container and further hinder sample biodegradation. Water samples in VOA vials with no headspace should not be frozen, but subsamples added to prepared headspace vials may be frozen, provided the integrity of the container seal is maintained. Freezing of soil samples is also appropriate provided the storage temperature is not lower than the minimum temperature recommended by the manufacturer for maintaining integrity of the container seal. Freezing in this temperature range may be used to extend the holding time of soils in sealed air-tight coring devices and in sealed headspace vials with reagent water, even if no other chemical preservative is added. See Table A1 in the Appendix of method 5035A for more details. The sample storage area should be free of organic solvent vapors.

8.5.2 All samples should be analyzed within 14 days of collection or sooner if labile compounds are target analytes. See the cautionary notes in Table 4-1 of Chapter Four, Method 5035, Appendix A, Table A-1, and the list of analytes in Sec. 1.1 of this method pertaining to certain compound classes and applicable preservation options that

may affect target analyte stability and analytical holding times. Samples not analyzed within this period should be identified to the data user and the results considered minimum values unless it can be demonstrated that the reported VOC concentrations are not adversely affected by preservation, storage and analyses performed outside the recommended holding times.

9.0 QUALITY CONTROL

9.1 Refer to Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. It should be noted that several methods (e.g., Method 8000) also contain general QC criteria and guidance that pertain to the individual methods referenced therein (e.g., Methods 8081, 8082, 8260 and 8270). Individual methods may also contain QC criteria specific only to that method. The QC criteria in the general methods take precedence over chapter QC criteria. Method-specific QC criteria take precedence over general method QC criteria.

Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Initial Demonstration of Proficiency (IDP)

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000D, Sec. 9.3 for information on how to accomplish a demonstration of proficiency.

9.3 Lower Limit of Quantitation (LLOQ) check standard

The laboratory shall establish the LLOQ as the lowest point of quantitation, which in most cases, is the lowest concentration in the calibration curve. LLOQ verification is recommended for each project application to validate quantitation capability at low analyte concentration levels. This verification may be accomplished with either clean control material (e.g., reagent water, solvent blank, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix, free of target compounds. Optimally, the LLOQ should be less than the desired regulatory action levels based on the stated DQOs.

In order to demonstrate the entire sample preparation and analysis process at the lower limit of quantitation (LLOQ), a LLOQ check standard (not part of an initial calibration) is prepared by spiking a clean control material with the analyte(s) of interest at the predicted LLOQ concentration level(s). Alternatively, a representative sample matrix may be spiked with the analytes of interest at the predicted LLOQ concentration levels. The LLOQ check is carried through the same preparation procedures as environmental samples and other QC samples.

Recovery of target analytes in the LLOQ check standard should be within established in-house limits, or other such project-specific acceptance limits, to demonstrate acceptable method performance at the LLOQ. Until the laboratory has sufficient data to determine

acceptance limits, the LCS criteria ± 20% may be used for the LLOQ acceptance criteria. This acknowledges the poorer overall response at the low end of the calibration curve. Historically-based LLOQ acceptance criteria should be determined as soon as practical once sufficient data points have been acquired. Additional information on LLOQ can be found in 8000D, Sec. 9.7.

9.4 Initially, before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the analysis of a method blank. As a continuing check, each time samples are extracted, cleaned up, and analyzed, and when there is a change in reagents, a method blank should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. If a peak is observed within the retention time window of any analyte that would interfere with measurement of that analyte, determine the source and eliminate it, if possible, before analyzing the samples. The blanks should be carried through all stages of sample preparation and analysis. When new reagents or chemicals are received, the laboratory should monitor method and/or instrument blanks associated with samples for any signs of contamination. It is not necessary to test every new batch of reagents or chemicals prior to sample preparation if the source shows no prior problems. However, if reagents are changed during a preparation batch, separate blanks must be prepared for each set of reagents.

The laboratory should not subtract the results of the method blank from those of any associated samples. Such "blank subtraction" may lead to negative sample results. If the method blank results do not meet the project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the method blank results, and a discussion of the corrective actions undertaken by the laboratory.

9.5 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, method sensitivity). At a minimum, each batch of 20 or fewer field samples should include at least one method blank, a laboratory control sample (LCS), and either a matrix spike/matrix spike duplicate (MS/MSD) pair or a matrix spike and duplicate analysis of one field sample. When used, surrogates may be added to each field sample and QC sample and their recovery monitored to evaluate the effect of the sample matrix. Any method blanks, matrix spike samples, and duplicate QC samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

See Methods 5000 and 8000 for procedures to follow to demonstrate acceptable continuing performance on each set of samples to be analyzed.

9.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.7 The laboratory should have quality control procedures to make sure that sample integrity is not compromised during the sample collection and sample handling process, e.g., through analysis of trip blanks, method blanks, etc. In addition, it would be advisable for the laboratory to monitor the internal standard (IS) area counts for all samples; leaks attributed to a poor seal with the vial caps and septa will be evident by low IS area counts. Sample containers and data results for instances where low IS area counts are observed and leaks are suspected should be discarded. Low area counts of the less volatile internal standards may also be attributed to matrix effects and should not be confused with a leaking vial.

9.8 Heating the sample/chemical preservative/matrix modifier mixture can exacerbate chemical interferences such as those introduced by acid catalyzed hydrolysis or base catalyzed substitution and elimination reactions. This can only be monitored through a matrix spike of a sample from every project analytical batch. The spiking solution should be the same as that used to prepare the calibration standards in order to minimize sources of variability in evaluating spike recovery. The acceptance criteria shall be those recommended in the determinative method or specified by a properly executed systematic planning document. If these criteria cannot be met, the analyst may adjust the pH of the mixture through the addition of solid $NaHSO_4$ to excessively basic mixtures or solid $Na_3PO_4 \bullet 12H_2O$ to excessively acidic mixtures. After this is done, the matrix spike analysis should be repeated with an unanalyzed vial. If the results are acceptable, this pH adjustment should be made to all samples in the appropriate analytical batch. Even if the pH-adjusted matrix spike analysis is acceptable, the data user must be made aware that the initial matrix spike failed and the pH adjustment was necessary. The results from the pH adjusted samples should be reported, and the data user must be made aware that the results for the analytes for which the initial matrix spike failed are questionable.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization and refer to the appropriate determinative method for additional calibration and standardization procedures.

11.0 PROCEDURE

11.1 Sample preparation - Sample preparation in the laboratory will be necessary except when a soil sample is collected and used only for screening purposes. The procedure for sample preparation depends upon the matrix of the sample and the target analyte concentration range. To minimize loss of VOCs from the samples or exchange of the vial headspace with the room air, add spiking solutions quickly to cold sample vials soon after removing from refrigerated storage and either reseal or place a new cap on top of the vial and apply slight pressure in between preparation steps.

CAUTION: Adding standard solutions (e.g., internal standards) to a sealed vial by puncturing the PTFE septum face with a microsyringe exposes the gas phase contents of the vial to the silicone material backing the septum. This material may absorb some of the gas phase VOCs in the vial, causing problems with calibration, measurement in samples, spike recovery, *etc.*, as a function of exposure time. This problem is generally worse for the higher molecular weight VOCs with high octanol-water partition coefficients, and this practice should be avoided or the vial caps should be exchanged for caps with un-punctured septa soon after spiking if these VOCs are analytes of interest.

11.1.1 Water samples - The preparation of water samples inevitably involves some sample manipulation and exposure to the laboratory atmosphere. Extreme caution should be exercised to minimize any volatilization of analytes out of the sample contents and into the laboratory atmosphere. The first precaution is to prepare the water samples immediately after removal from cold storage. The decreased temperature reduces analyte volatility, and the benefits of this are substantially greater than the inaccuracies introduced by measuring sample volume at lower temperatures.

11.1.1.1 Add 5 mL of the matrix modifier solution to a headspace vial (Sec. 6.0), if used. Otherwise, add 5 mL reagent water. Set the septum and crimp top onto the vial and move the crimping tool to a readily available position.

11.1.1.2 Insert the tip of a 5-mL gas tight syringe through the septum of the vial to withdraw the sample. Fill the syringe, taking care to prevent air from leaking into the syringe while filling it, then remove the syringe from the sample and place it in the liquid phase in the headspace vial. Inject the entire aliquot into the headspace vial, then quickly add the internal standard and/or surrogate standard solution, if used, and immediately seal the vial. This process of taking an aliquot destroys the validity of the liquid sample for future analysis. Therefore, if there is only one VOA vial, the analyst should prepare a second sample in the same manner as the first at this time to protect against possible loss of sample integrity. This second sample is stored at <6°C until the analyst has determined that the first sample has been analyzed properly. If a second analysis is needed, it should be completed within 24 hr.

11.1.2 Soil samples - If the sample will be analyzed by direct vapor partitioning for low level analysis, follow the instructions in this section. If the sample will be extracted with solvent and the extract diluted for high level analysis, proceed to Sec.11.4.

11.1.2.1 If the soil sample was placed into a headspace vial with neither water nor matrix modifier and the sample mass was not recorded in the field, estimate the sample mass by weighing the vial plus soil and subtract the mass of an empty vial and cap. Then, unseal the vial, add 10.0 mL of matrix modifying solution, if used, or reagent water, along with any internal standard and/or surrogate standard used, and immediately reseal the vial. As noted in Sec. 8.0, VOC losses may occur as a result of opening the vial and displacing 10 mL of headspace.

CAUTION: Only open and prepare one vial at a time to minimize loss of volatile organics.

11.1.2.2 If the soil sample was placed into a headspace vial with reagent water or the matrix modifier solution at the time of sampling, first weigh the sealed vial and its contents to 0.01 g. If the matrix modifying solution was added at the time of sampling (Sec. 8.3.1), the tare weight does not include 10 mL of matrix modifying solution. Therefore, weigh the field blank associated with those samples and subtract from it the tare weight of the vial in which the field blank was prepared. Use the difference as the weight of the matrix modifying solution in the samples. (Although this approach may introduce some error into the sample results, that error should be much less than the changes that will occur in an unpreserved sample shipped to the laboratory without the modifier). If surrogates and/or internal standards were not added at the time of sampling, they should be added at this time.

11.2 The low-concentration method utilizing an equilibrium headspace technique is found in Sec. 11.3 and sample preparation for the high-concentration method is found in Sec. 11.4. The high-concentration method is recommended for samples that obviously contain oily material or organic sludge waste (see Sec. 4.4). See Method 8000 for guidance on the selection of a GC or GC/MS determinative method. For the analysis of gasoline, use Method 8021 with GC/PID (photoionization detector) for BTEX (benzene, toluene, ethylbenzene, and xylenes) in series with Method 8015 with the GC/FID (flame ionization detector) detector for other gasoline components. If GC/MS analysis is preferred, follow Method 8260. For the analysis of MTBE and

the other fuel oxygenates, use either Method 8015 with the GC/FID detector or Method 8260 using GC/MS.

11.3 Low-concentration (direct vapor partitioning) method for water, soil/sediment and solid waste amenable to the equilibrium headspace method.

11.3.1 Calibration

Prior to using this introduction technique for any GC or GC/MS method, the system must be calibrated. General calibration procedures are discussed in Method 8000, while the determinative methods and Method 5000 provide specific information on calibration and preparation of standards. Normally, external standard calibration is preferred for the GC methods because of possible interference problems with internal standards. If interferences are not a problem, based on historical data, internal standard calibration is acceptable. The GC/MS methods normally utilize internal standard calibration. The GC/MS methods require instrument tuning prior to proceeding with calibration.

11.3.1.1 GC/MS tuning

If a GC/MS determinative method is employed, prepare a headspace vial containing reagent water and the amount of 4-bromofluorobenzene (BFB) listed in the determinative method.

11.3.1.2 Initial calibration

Prepare a minimum of five headspace vials for calibration standards, as described in Sec. 7.6, and a reagent blank (Sec. 7.5), and proceed according to Sec. 11.3.2 and the determinative method selected. The mixing step is unnecessary, because no soil is present in the vial. See method 8000D for the minimum number of calibration standards recommended for each type of calibration model.

11.3.1.3 Calibration verification

Prepare a headspace vial, as described in Sec. 7.6, by spiking with the mid-concentration calibration standard. Proceed according to Sec. 11.3.2.1 (beginning by placing the vial into the autosampler) and the determinative method. If a GC/MS determinative method is employed, prepare a second headspace vial containing reagent water and the amount of BFB listed in the determinative method.

11.3.2 Headspace analyzer operating conditions

The conditions described throughout Sec. 11.3 were experimentally optimized using the equipment described in Reference #1 in Sec. 16 and employing Method 8260 as the determinative method. If other headspace systems and determinative methods are utilized, it is recommended that the manufacturer's headspace operating conditions be followed, provided that they are appropriate for the determinative method to be employed.

11.3.2.1 Mix the samples (on a rotator or shaker) for at least 2 min. For samples that contain water insoluble materials, care must be exercised during mixing to prevent this material from adhering to the inner surface of the vial seal; otherwise the sampling needle can become contaminated with this material upon puncturing the seal. Care must also be exercised to avoid over filling the vial to prevent contaminating the needle with aqueous sample.

Place the vials in the autosampler carrousel at room temperature. The individual vials are heated to 85 °C and allowed to equilibrate for 50 min. Each sample is mixed by mechanical agitation during this equilibrium period. Each vial is pressurized with helium carrier gas to a minimum pressure of 10 psi.

11.3.2.2 A representative and reproducible sample of the pressurized headspace is transferred to the GC column through a heated transfer line according to the manufacturer's instructions.

11.3.2.3 Proceed with the analysis as per the determinative method of choice.

NOTE: If maintaining a specified pH is critical to quality assured measurement of the analyte(s) of concern (Sec. 4.7), the pH of each sample should be verified. If basic preservation is necessary, the pH of the sample should be verified to be ≥10 (see Sec. 7.8.1). If acid preservation is necessary, the pH should be verified to be ≤ 2, (see Sec. 7.8.2). This check may be performed after analysis of the sample in order to avoid compromising sample integrity. Wide-range pH paper should provide sufficient information to verify efficacy of the preservative.

11.4 High-concentration soil method

11.4.1 If the sample was collected as described in Sec. 8.3.2 without the addition of methanol to the vial, then weigh the sample to the nearest 0.01 g. Add twice the volume of methanol as the nominal sample mass to a tared VOA vial and immediately reseal the vial. Open only one vial at a time to minimize loss of VOCs. If the sample was collected in a sealable coring device as described in Sec. 8.3.3, add the methanol to a vial first, weigh the vial with the methanol and the cap together to obtain the tare mass, and then add the soil plug, seal immediately, reweigh, and calculate the sample mass.

11.4.2 If the procedure in Sec. 8.3.1 was employed for sample collection and either the matrix modifying solution or organic-free reagent water was added to the sample vials, subsamples for high concentration analysis should be taken from the separate VOA vials collected without matrix modifying solution or reagent water as described in Sec. 8.3.2 or from the vials collected for dry solids determination. Transfer approximately 5 g of sample from the 40 or 60 mL VOA vial into a tared VOA vial containing 10.0 mL of methanol, seal the vial, and reweigh to estimate the mass of sample transferred. Open only one vial at a time to minimize the loss of volatile organics. Substantial VOC losses may occur as a result of transferring a subsample from one vial to another using this procedure. See Sec. A.5 in the Appendix of Method 5035A for more details.

11.4.3 Mix by shaking for 10 min at room temperature. Decant 2 mL of the methanol extract to a screw-top vial with PTFE-faced septa and seal. Withdraw 10 µLand inject into a headspace vial containing 10.0 mL of matrix modifying solution or organic free reagent water. A larger volume of methanol may be added provided the methanol content does not adversely affect the analyte responses (refer to Sec. 7.4.1). Add internal standards and/or surrogates as appropriate, and analyze by the headspace procedure by placing the vial into the autosampler and proceeding with Sec. 11.3.2.1.

12.0 DATA ANALYSIS AND CALCULATIONS

There are no data analysis and calculation steps directly associated with this procedure. Follow the directions given in the determinative method.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance goals should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

13.2 Water samples

This method was used to measure several VOCs in groundwater samples. The samples were collected from two sites: twenty-four samples were collected from the first site (site A) and twenty-three samples were collected from the second site (site B). Using a basic preservative to prevent the hydrolysis of ethers such as MTBE, multiple groundwater vials were collected at each sampling point. The samples were analyzed by three independent laboratories. All of the laboratories used this method for sample preparation, and each laboratory used a different determinative method. One laboratory used a GC/MS technique with a quadrupole mass spectrometer (Method 8260), another used a GC/MS technique with an ion-trap mass spectrometer (Method 8260), and the third used a GC/FID technique (Method 8015). The example results of the analyses are shown in Figures 1 through 6. Since all three laboratories followed the same project plan and the same data quality objectives, the data generated by the three laboratories is mutually comparable, even though they used different techniques. As recommended in Sec. 9.8, matrix spike studies were done at each site. The example percent recoveries from the site A studies are shown in Figure 7, while those from site B are shown in Figure 8. Figure 8 shows that one of the labs had poor recovery for MTBE. However, the recovery of the other ethers was acceptable, indicating that hydrolysis was unlikely to be the source of the problem. The effect was attributed to sample matrix interference.

13.3 Soil samples - Single-laboratory accuracy and precision data were obtained for the method analytes in two soil matrices, i.e., sand and garden soil. These data are found in Tables 26-28 of Method 8260C.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety,
http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult the ACS publication listed in Sec. 14.2.

16.0 REFERENCES

1. P. Flores, and T. Bellar, "Determination of Volatile Organic Compounds in Soils using Equilibrium Headspace Analysis and Capillary Column Gas Chromatography/Mass Spectrometry," U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring Systems Laboratory, Cincinnati, OH, December, 1992.

2. B. V. Loffe, and A. G. Vitenberg, "Headspace Analysis and Related Methods in Gas Chromatography," John Wiley and Sons, 1984.

3. R. J. Pirkle, and R. P. McLoughlin, "The Analysis of Selected Components of Reformulated Gasoline in Environmental Samples" *from* MTBE Handbook, *ed*. Kostecki, P. and Moyer, E. Amherst Scientific Publishers, 2002.

4. USEPA OUST, *Environmental Fact Sheet: Analytical Methods for Fuel Oxygenates,* EPA 510-F-03-001, April, 2003.

5. H. White, B. Lesnik, and J. T. Wilson, "Analytical Methods for Fuel Oxygenates", *LUSTLine* (Bulletin #42), October, 2002, http://www.epa.gov/oust/mtbe/LL42Analytical.pdf.

6. RCRA Organic Methods Workgroup Meeting Minutes, March 20, 2012.

7. RCRA Organic Methods Workgroup Meeting Minutes, March 22, 2012.

8. RCRA Organic Methods Workgroup Meeting presentation describing changes to Method 5021, "Proposed Changes to SW-846 Method 5021A, VOCs by Static Headspace", March 1, 2012.

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method.

FIGURE 1

EXAMPLE RESULTS FOR SITE A STUDY OF ETHYL *TERT*-BUTYL ETHER

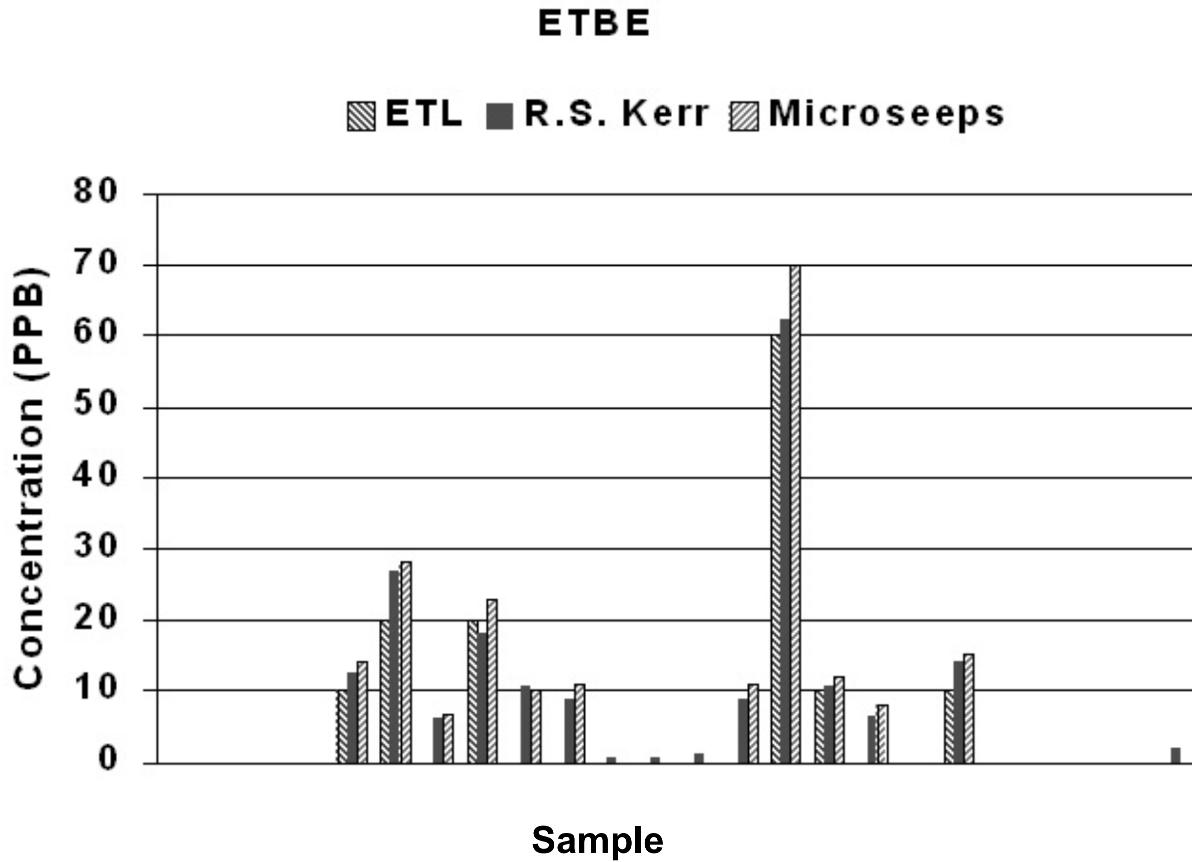


FIGURE 2

EXAMPLE RESULTS FROM SITE A STUDY FOR *TERT* AMYL METHYL ETHER

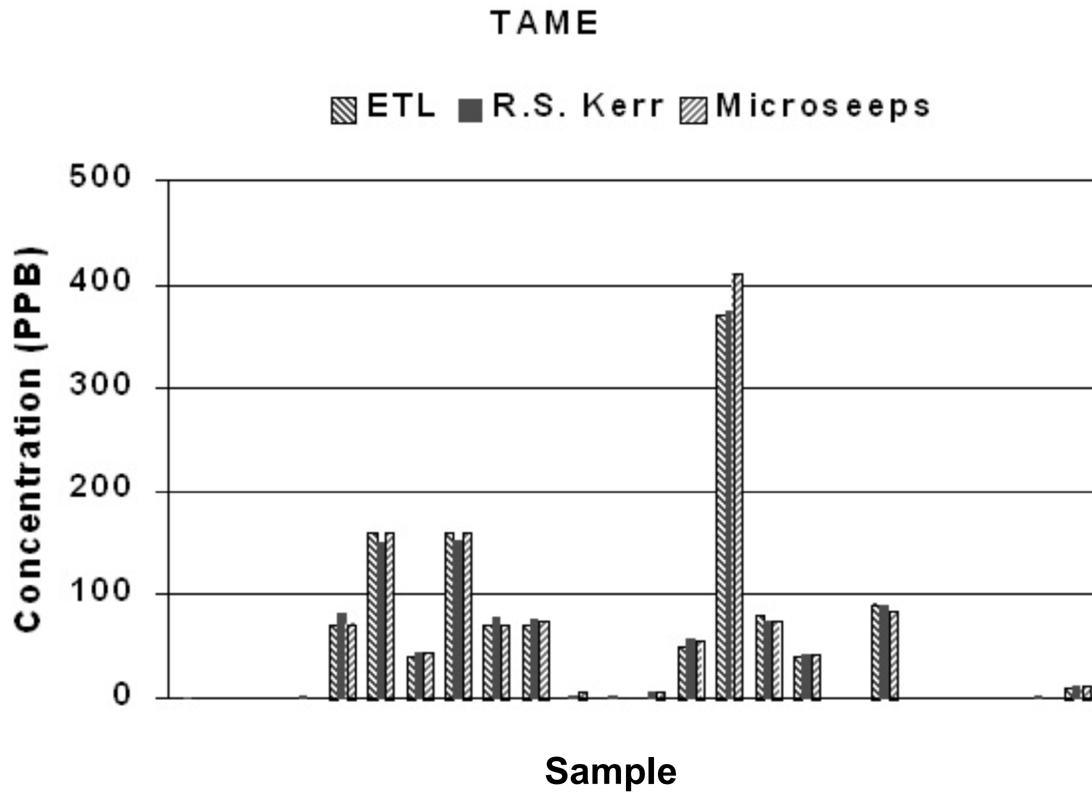


FIGURE 3

EXAMPLE RESULTS FROM SITE A STUDY FOR METHYL *TERT*-BUTYL ETHER

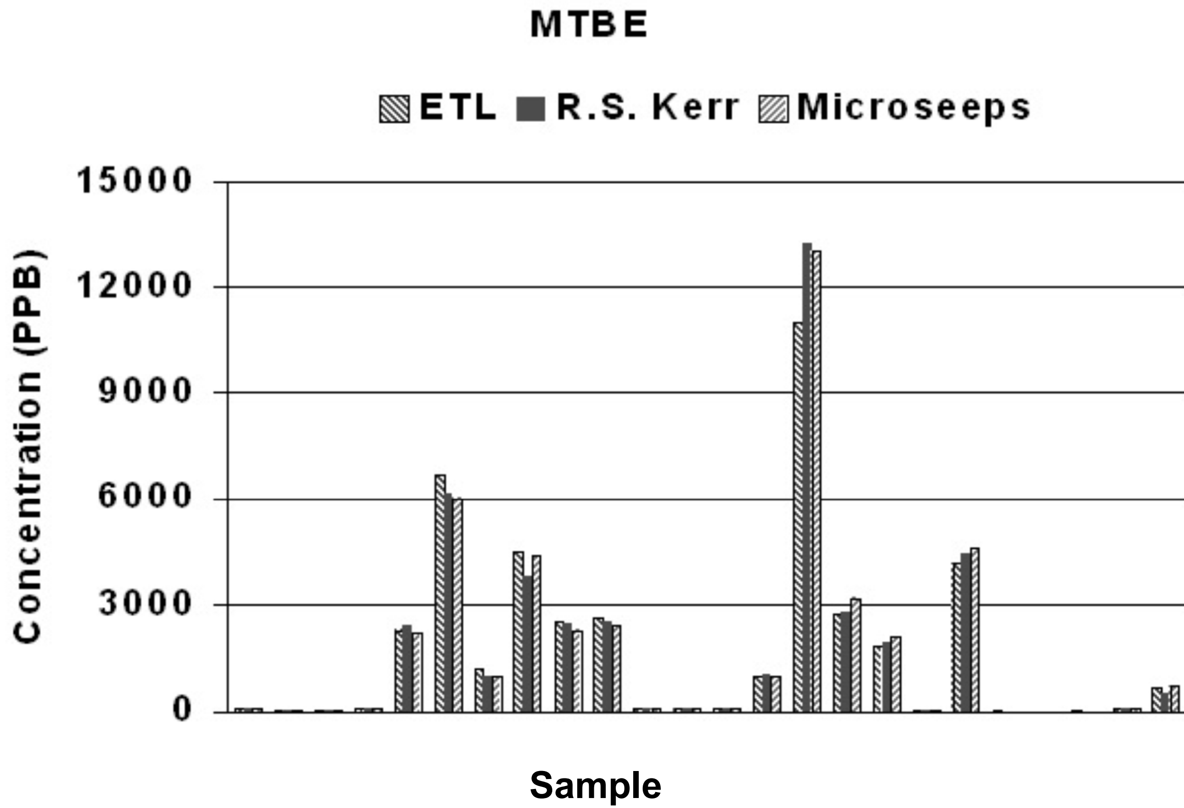
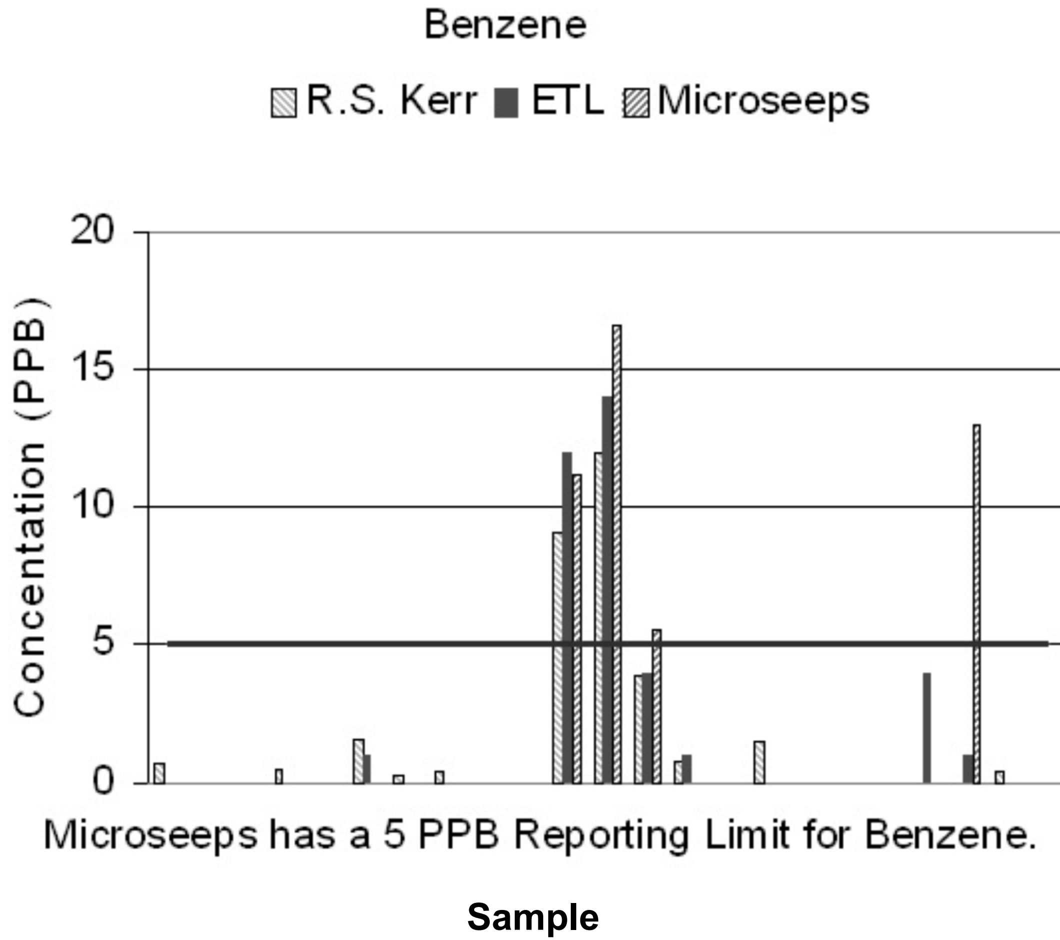


FIGURE 4

EXAMPLE RESULTS FROM SITE B STUDY FOR BENZENE



Sample

FIGURE 5

EXAMPLE RESULTS FROM SITE B STUDY FOR METHYL *TERT*-BUTYL ETHER

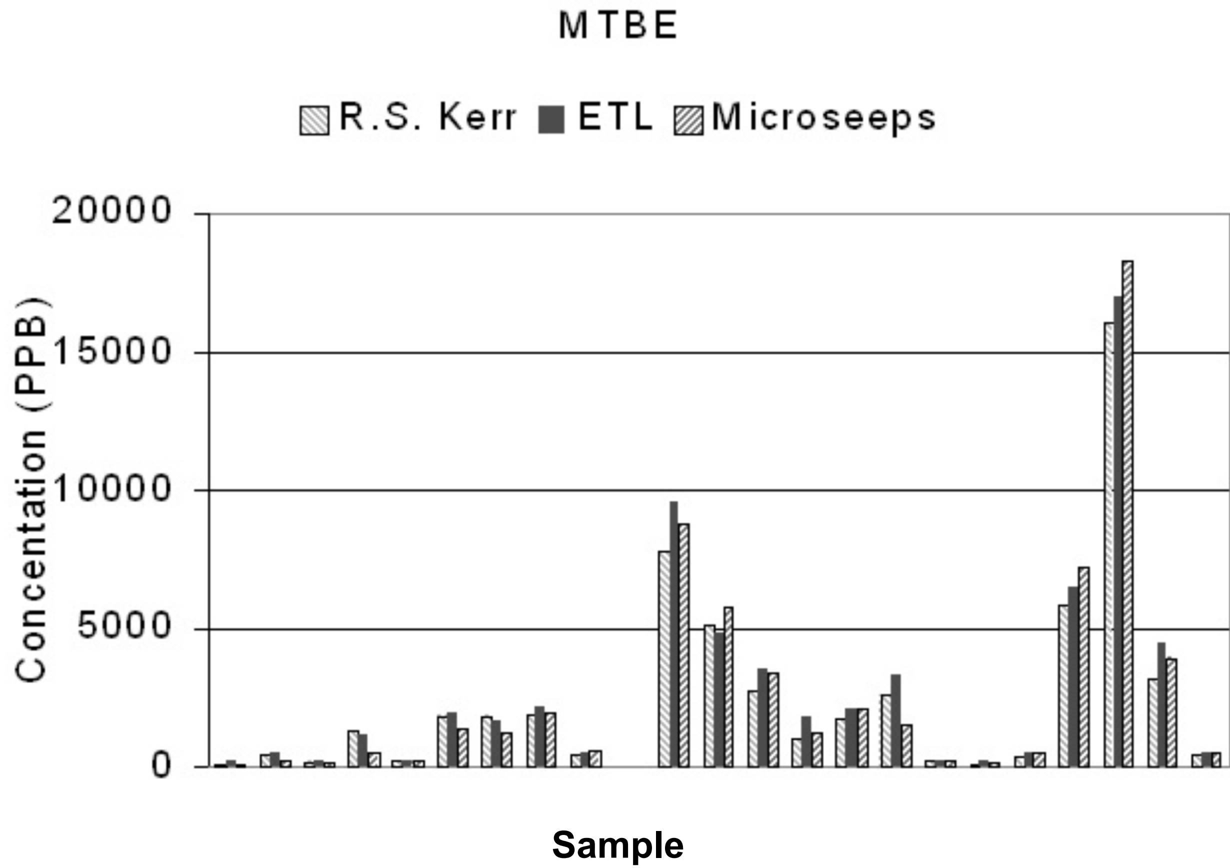


FIGURE 6

EXAMPLE RESULTS FROM SITE B STUDY FOR *TERT*-BUTYL ALCOHOL

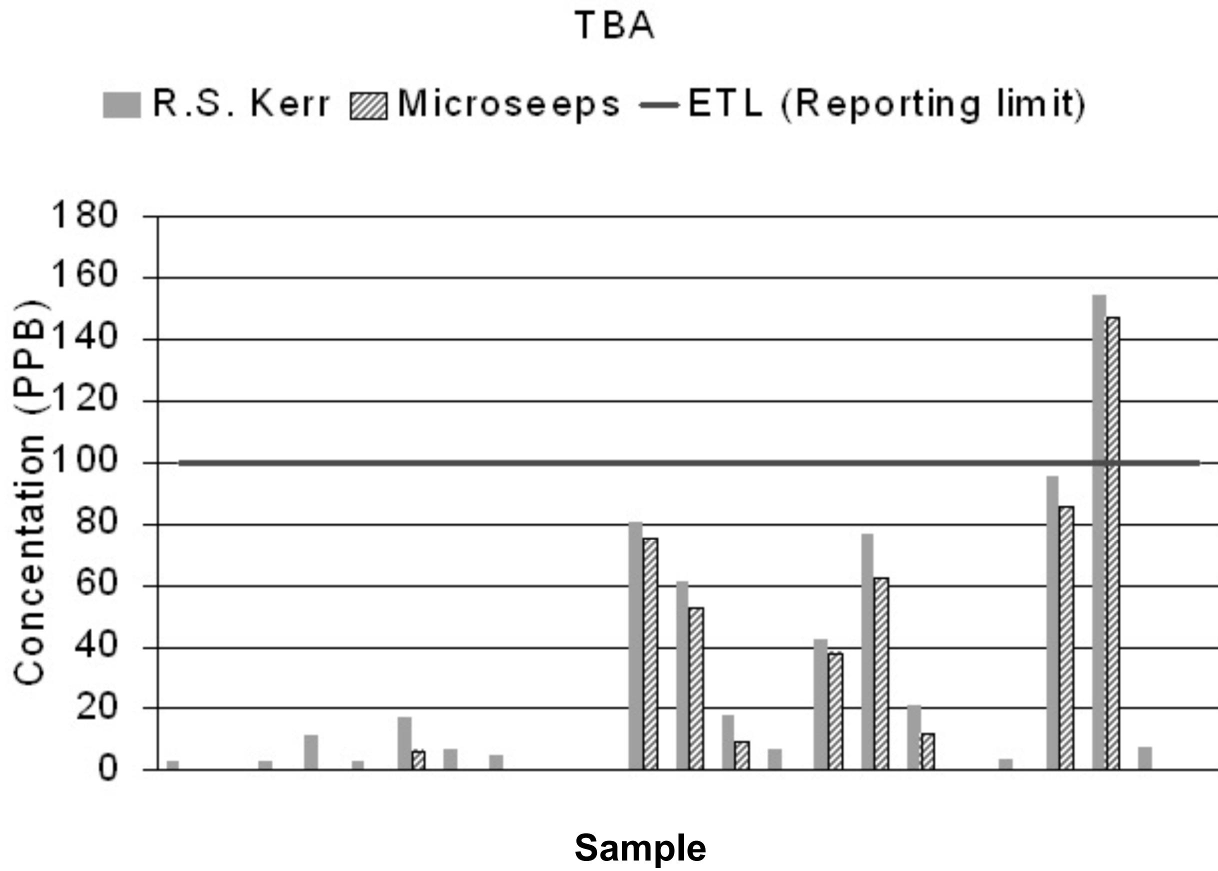


FIGURE 7

EXAMPLE PERCENT RECOVERIES FROM THE MATRIX SPIKE STUDIES OF SITE A

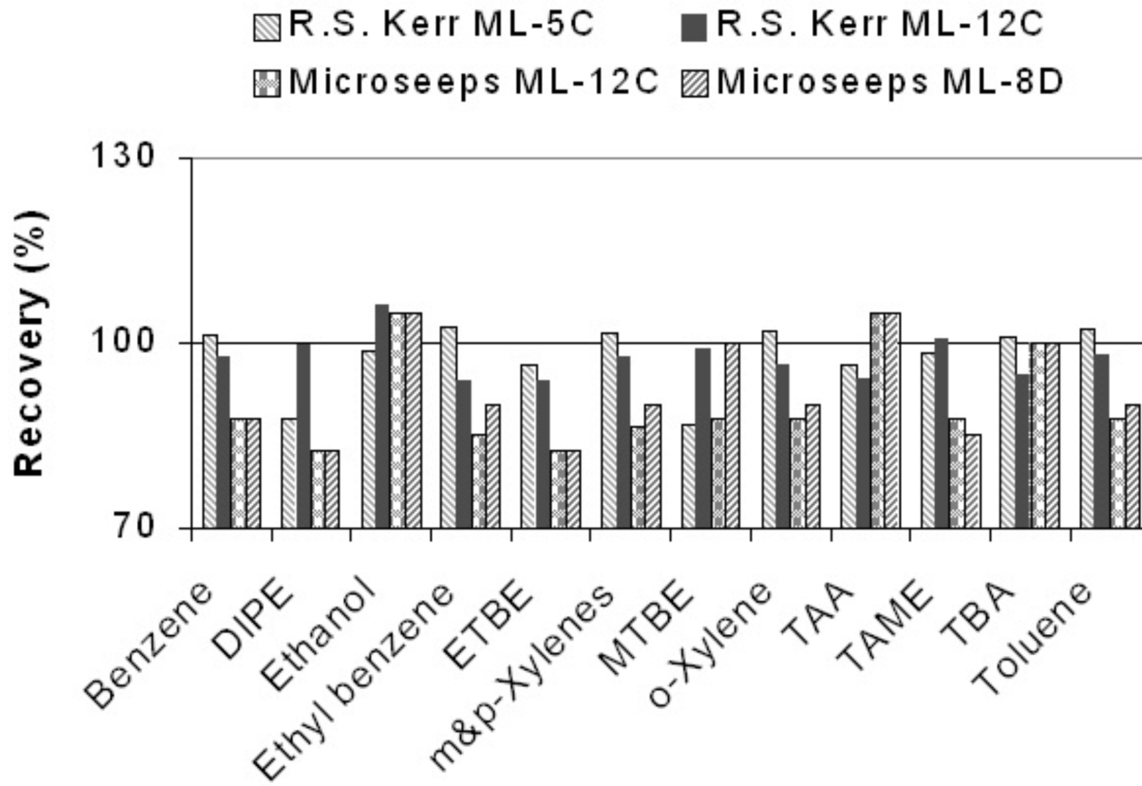
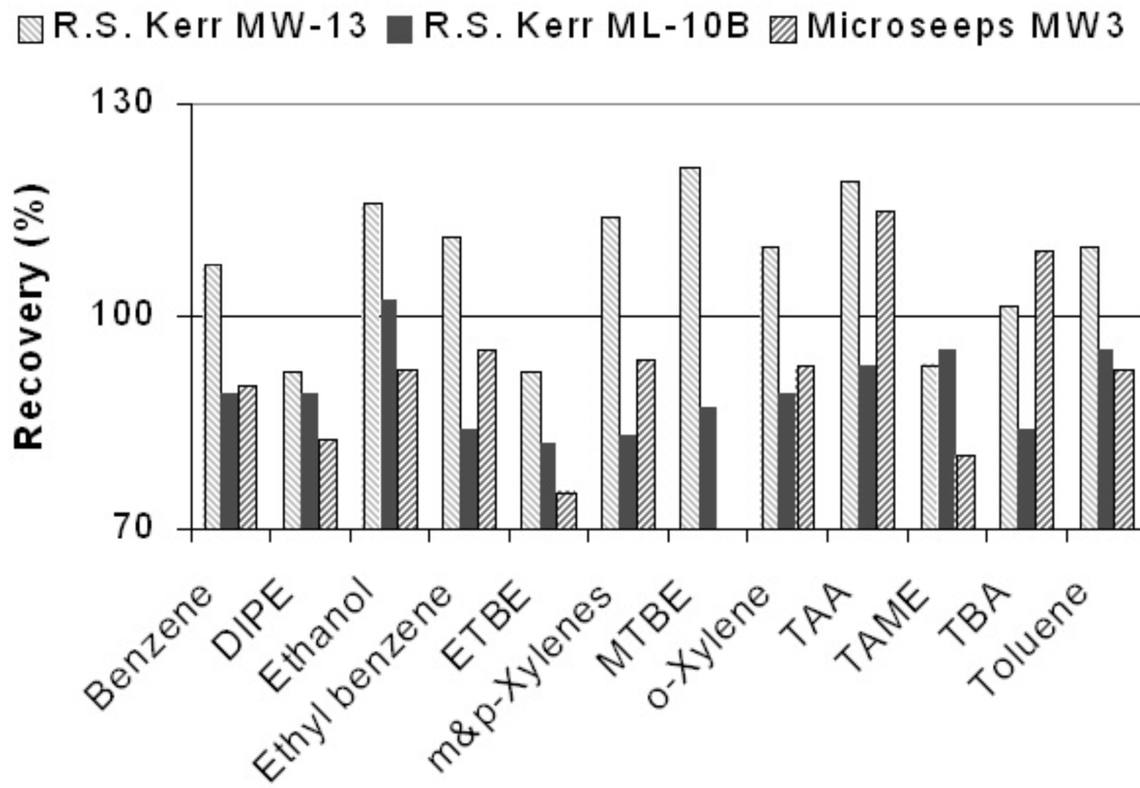


FIGURE 8

EXAMPLE PERCENT RECOVERIES FROM THE MATRIX SPIKE STUDIES OF SITE B



Appendix A:

Summary of Revisions to Method 5021A (as compared to previous Revision 1, June 2003)

1. Improved overall method formatting for consistency with new SW-846 methods style guidance. The format was updated to Microsoft Word .docx.
2. Minor editorial and technical revisions were made throughout to improve method clarity.
3. The revision number was changed to 2 and the date published was changed to July 2014.
4. This appendix was added showing changes from the previous revision.
5. Added updated IDP language and LLOQ verification standard language to Sections 9.2 and 9.3.
6. Included response column and a classification system for analytes in Secs. 1.1 and 1.2 to provide an indication of which VOC responses were improved by the matrix modifier.
7. Added a sealable, air-tight coring device as an alternative sample collection option for soils to Sec. 8.3.3.
8. Added an alternative for calibration standard preparation to Sec. 7.4.1 that allowed for multiple calibration levels prepared by adding different volumes of one or more stock solutions.
9. Clarified in Sec. 1.4 the major sources of measurement bias expected for sample analysis using this method, as well as which sources of measurement bias the matrix modifier may improve, which sources of measurement bias may be made worse by the matrix modifier, and under what other circumstances not adding the matrix modifier may be appropriate.
10. Added a caution after Sec. 11.1 regarding the expected effect of compromising the PTFE face of a vial seal on recovery of oil soluble target analytes.

Appendix C. Cannabis Science Task Force Members

Steering Committee

- Annette Hoffmann Ph.D., Ecology, Environmental Assessment Program (EAP) Manager, and Committee Chair
- Jessica Archer, Ecology, EAP Section Manager
- Shelly Rowden, DOH
- Allyson Clayborn, DOH
- Brad White, WSDA
- Kendra Hodgson, WSLCB
- Amber Wise, Medicine Creek Analytics, representing the Puyallup Tribe of Indians
- Nick Mosely, Confidence Analytics
- Jeff Doughty, Capitol Analysis Group
- Sara Sekerak, Ecology, Lead Task Force Chemist, interim Proficiency Testing Lead (non-voting member)
- Nicholas Poolman, WSLCB chemist (non-voting member)
- Mike Firman, WSDA chemist, Potency, Moisture content and Water Activity Workgroup Lead (non-voting member)
- Raymond Gee, DOH microbiologist, Microbiological and Mycotoxins Workgroup Lead (non-voting member)
- Caroline West, DOH chemist, Heavy Metals Workgroup lead (non-voting member)
- Ryan Zboralski, Ecology chemist and Proficiency Testing, and Residual Solvents and Mycotoxins Lead (non-voting member)

Potency, Moisture Content, and Water Activity Workgroup

- **Mike Firman, WSDA, workgroup lead**
- Ben Hart, Testing Technologies
- Lawrence Bowman, Capitol Analysis Group
- Chris Johnson, Medicine Creek Analytics
- Cristi Crofton, Confidence Analytics
- Steve Officer, DOH
- Nicholas Poolman, WSLCB
- Ryan Zboralski, Ecology
- Sara Sekerak, Ecology

Heavy Metals Workgroup

- **Caroline West, DOH, workgroup lead**
- Srinivasa Reddy Mallampati, Medicine Creek Analytics

- Tania Sasaki, Confidence Analytics
- Tim McCall, Dragon Analytical
- Curtis Deer, Praxis
- Mike Firman, WSDA
- Nicholas Poolman, WSLCB
- Sara Sekerak, Ecology

Proficiency Testing Workgroup

- **Ryan Zboralski, Ecology, workgroup lead**
- Kyle Shelton, Medicine Creek Analytics
- Steven Loague, Integrity Labs
- James Burns, Treeline
- Bonnie Luntzel Praxis
- Steve LaCroix, DOH
- Qingfen Gu, WSDA
- Nicholas Poolman, WSLCB
- Sara Sekerak, Ecology

Residual Solvents and Mycotoxins[44] Workgroup

- **Ryan Zboralski, Ecology, workgroup lead**
- Damien Gadomski, Pacific Botanicals
- Kyle Shelton, Medicine Creek Analytics
- Tania Sasaki, Confidence Analytics
- Steven Loague, Integrity Labs
- Mike Firman, WSDA
- Nicholas Poolman, WSLCB
- Sara Sekerak, Ecology

Microbiological (and Mycotoxins[45]) Workgroup

- **Raymond Gee, DOH, workgroup lead**
- Tim Casad, Pacific Botanical
- Lauren Christiansen, Medicine Creek Analytics
- Tori Wallen, Confidence Analytics
- Carol Larson, WSDA
- Crystal Verellen, WSDA
- Nicholas Poolman, WSLCB
- Sara Sekerak, Ecology

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[44] Mycotoxins by liquid chromatography mass spectrometry.
[45] Mycotoxins by biological assay, as time allows.

Appendix D. Cannabis Matrix Proficiency Testing Trial

The Proficiency Testing (PT) Workgroup explored three cannabis-matrix pathways during the tenure of the Cannabis Science Task Force (CSTF). The three pathways included:

1. An in-state PT program which requires a state entity[46] to become a PT provider to produce cannabis matrix PTs for high priority analyte/matrix combinations (or fields of testing).
2. State entity[47] oversees a third-party independent PT provider activity, allowing for this provider to come into Washington and create PT samples using state-supplied cannabis.
3. State entity[48] provides technical oversight for existing out-of-state PT providers/ programs[49].

One or a combination of these pathways were considered capable of filling the gap of cannabis-matrix PT for the Washington State cannabis testing industry.

Cannabis matrix proficiency testing (PT) trial development using an independent provider

The PT Workgroup focused its work on pathway 2 after conversations with Oregon and Colorado about implementation of similar programs in their states, using an ISO/IEC 17043 and ISO 17034 compliant provider, Phenova Inc.[50] The PT Workgroup created a proof-of-concept trial study to run through the complicated logistics of a third-party independent PT provider (1) coming into the state, (2) sourcing of a legal quantity of cannabis flower, and (3) producing and distributing cannabis PT samples.

The PT Workgroup presented the CSTF with tasks to be accomplished before allowing a trial study with Phenova to begin. The workgroup's aim was to (1) collect information from the proof-of-concept study and (2) incorporate that information to develop comprehensive recommendations for the in-state cannabis matrix PT program using a third-party provider in Washington. The workgroup made a best attempt to formulate the study around how they understood state laws and WSLCB rules. All law and rule interpretation remains under the WSLCB's authority. Law and rule clarifications were delivered to the CSTF through the WSLCB representative.

[46,47,48] State entity must remain separate from Ecology's Laboratory Accreditation Unit (LAU). This distinction is necessary to ensure the integrity and facilitation of independent and impartial accreditations, i.e., LAU cannot participate in PT program (and sample) design and also be the judge of laboratory proficiency when used.
[49] For minimal selective non-critical PT samples (i.e. microbiological PT using hemp).
[50] Ty Garber from Phenova provided a presentation and question/answer session with the Task Force on November 15, 2019; slides: https://www.ezview.wa.gov/Portals/_1962/Documents/CannabisSTF/PhenovaPTPresentation.pdf, recording: https://www.youtube.com/watch?v=haCkgPwsiW4.

The PT Workgroup presented their major findings to the CSTF. Several challenges were identified, and no motions were passed to allow the proof-of-concept trial to materialize.

Identified PT provider for trial: Phenova, Inc.

- Meets needs: Phenova willing to travel into Washington to manufacture a PT samples with in-state acquired cannabis and cannabis products.
- ISO/IEC 17043 and ISO 17034 compliant.
  - Is a current approved third-party provider for cannabis "surrogate" PT samples and a current approved provider of environmental PT samples in Washington.
- Experience: Phenova currently performs successful in-state cannabis-matrix PT production and distribution for Oregon and Colorado cannabis testing industries, and is beginning to work with Nevada.
- Only PT provider to respond to CSTF inquiry to discuss their operation.
- Trial run performed at no cost to laboratories (CSTF funds to be used for purchase of cannabis).

Hosting location: Washington State Department of Agriculture (WSDA) Chemical and Hop Laboratory

- The WSDA laboratory location is independent of the labs performing PT. This limits potential conflicts if the host were a certified testing lab participating in the study (e.g., questions of impartiality; multi-day business interruption; separate space and resources required by trial provider).

  Note: WSLCB was not comfortable with a certified lab, producer, or processor location to perform the trial; this was not explored further after WSDA agreed to host.[51]
- The WSDA laboratory is already performing cannabis testing for pesticides through an interagency agreement (IAA) with WSLCB. Cannabis is permitted on WSDA premises for testing purposes, and the existing IAA could be expanded for this PT production purpose.
- The WSDA laboratory has a full-service lab with instrumentation and methods to perform ISO 17034 homogenization testing.
- CSTF funds pay (through an Ecology-WSDA IAA) for WSDA laboratory use. This may include paying for instrument preparation, chemist time, chemicals and reagents, hazardous waste disposal, cannabis disposal, and potential storage of extra PT material. CSTF funds also pay for administrative hosting incidentals.

[51] It was mentioned that WAC 314-55-1025(6) may limit certified laboratories from hosting; however, this was not explored in detail by the workgroup.

Licensing requirements

- The Phenova representative did not need a license to acquire the cannabis from a licensed retail location, as he would stay under the one-ounce possession and carry limit for the small trial.
  - Larger trials and future studies (more participants, more matrix/analyte PTs generated) would need a pathway[52] or license option for a PT provider to acquire and possess larger quantities of cannabis materials.
- No additional transporting license would be necessary as manufactured PT samples could be transported to labs by already existing licensed transporters.
  - Phenova was still considered to be providing the service for monitory gain, which is not permitted without a license. A PT provider does not fit into the current license types (i.e., retailer, producer, processor, transportation, or cannabis research). To add a new license type, legislation would need to be passed.

Traceability of cannabis

- Cannabis material sourced or purchased for PT would be removed from the WSLCB LEAF tracking system; therefore, PT samples generated from these materials would not be included in the LEAF tracking system for testing/tracking.
- WAC 314-55-1025(6) does not create a clear pathway for labs to test cannabis-containing PT samples.

[52] The Oregon Liquor and Cannabis Commission provides Phenova with a waiver letter to purchase cannabis materials higher than Oregon's legal limit for personal use in order to facilitate PT studies and PT sample production.